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(54) Title: HUMAN SIGNAL TRANSDUCTION SERINE/THREONINE PROTEIN KINASE

(57) Abstract

An isolated and purified human signal transduction serine threonine protein kinase is described. A cDNA sequence which encodes the native signal transduction polypeptide is disclosed as well as the structural coding region and the amino acid residue sequence of the protein kinase. Methods are provided which employ the novel sequences to identify compounds that modulate the biological and/or pharmacological activity of the signal transduction protein kinase and hence regulate cellular and tissue physiology. Biologically-effective antisense molecules, as well as dominant negative mutant versions of the protein kinase, are described which are suitable for therapeutic use. The invention is also related to the diagnosis, study, prevention, and treatment of pathophysiological disorders related to or mediated by the signal transduction molecule.

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HUMAN SIGNAL TRANSDUCTION SERINE/THREONINE PROTEIN KINASE

Field Of The Invention

The present invention relates to nucleic acid and amino acid sequences pertaining to a novel human signal transduction serine threonine protein kinase and to the use of these sequences to identify compounds that modulate signal transduction activity of the native biomolecule. The invention also relates to biologically-effective antisense molecules, as well as dominant negative mutant versions of the protein kinase which are suitable for therapeutic use. The invention is also related to the diagnosis, study, prevention, and treatment of pathophysiological disorders related to signal transduction.

Background of the Invention

Kinase signal transduction pathways are biologically evolved devices which effectively transmit precise information via specific amplification cascades to the cell nucleus in order to trigger distinct gene expression in response to external, intercellular, as well as intracellular stimuli. Information is *transduced* by a series of accurate independent sequential covalent modifications to the nucleus, *via* distinct phosphorylation steps of independently specific cognate cytosolic biomolecules. Protein phosphorylation, by means of protein kinases, is now acknowledged as the most important means of acute regulation of protein function, and gene expression in eukaryotic cells. Intracellular biomolecule phosphorylation *via* specific kinases is ultimately responsible for *switching* of cellular activity from one state to another. There are two main subdivisions within the superfamily: the protein-serine/threonine kinases and the protein-tyrosine kinases. *Protein Phosphorylation*, Hardie, D. G., Oxford Press (1993).

Mitogen Activated Kinases (MAPK)

The control of cell development is a highly regulated process that responds to a number of physiological stimuli in the human body. Differentiation, proliferation, growth arrest, and apoptosis of cells depends on the presence of appropriate cytokines and their receptors, as well as the corresponding cellular kinase signal transduction cascades. Hu, Mickey C.T., *et al.*, Genes & Development, 10:2251 (1996); Grunicke, Hans H., *Signal Transduction Mechanisms in Cancer*, Springer-Verlag (1995). *See also*, Suchard, S.J., *et al.*, *Mitogen-Activated Protein Kinase Activation During IgG-Dependent Phagocytosis in Human*

Neutrophils, J. Immunol., 158:4961 (1997). One such protein kinase cascade, known as the *mitogen-activated protein kinase* (MAPK) cascade, is activated as an early event in the response of leukocytes, for example, to various stimuli. Stimulation of this pathway has been observed during growth factor-induced DNA synthesis, differentiation, secretion, and metabolism. The MAPK pathway has a critical role in the transduction of receptor-generated signals from the membrane to the cytoplasm and nucleus. Graves, J. D., *et al., Protein Serine/Threonine Kinases of the MAPK Cascade*, Annals New York Academy of Sciences, 766:320 (1995). It has been established that sustained activation of the MAPK cascade is not only required, but it is sufficient to trigger the proliferation of some cells and the differentiation of others. Cohen, P., *Dissection of Protein Kinase Cascades That Mediate Cellular Response to Cytokines and Cellular Stress*, Advances in Pharmacology, Academic Press, Hidaka, H., *et al.*, Eds., Vol. 36, 15 (1996); Marshall, C.J., Cell, 80:179 (1995). MAPKs are considered to be valuable pharmacological targets within the growth factor signaling pathways. Hidaka, H., *et al., Intracellular Signal Transduction*, Advances in Pharmacology, Academic Press (1996).

Stress Activated Protein Kinases (SAPKs)

Recent evidence suggests that cellular response to stress is controlled primarily through events occurring at the plasma membrane. Regulatory information is transduced via *stress activated protein kinases* (SAPKs) overlapping significantly with those important in initiating mitogenic responses. Stress responses represent carefully devised cellular defense mechanisms which were developed at an early point during evolution; evidenced by the fact that biomolecules implicated in stress response exhibit remarkable similarity across the animal kingdom. Welch, W. J., *et al., The Stress Response and the Immune System, Inflammation*: Basic Principles and Clinical Correlates, Raven Press, Gallin, J.I., *et al.*, Eds., Second Edition, 41:841 (1992). Exposure of cells to biological, chemical, or physical stress agents evokes a series of events leading to the activation of a wide group of genes including transcription factors as well as other gene products that are also rapidly and highly induced in response to mitogenic stimulation. Considering, *inter alia*, that stress has also been implicated in oxidant injury, atherosclerosis, neurogenerative processes, and cell senescence, elucidation of the components of mammalian stress-induced pathways should provide more specific targets that can be exploited therapeutically. N. J. Holbrook, *et al., Stress Inducible Cellular Responses*,

273, U. Feige, et al., Eds., Birkhauser Verlag (1996). Kyriakis, John M., *et al.*, *Sounding the Alarm: Protein Kinase Cascades Activated by Stress and Inflammation*, JBC, 271:40, 24313 (1996).

STE20

Ste20 represented the first identified member of a new family of stress activated serine/threonine protein kinases. Leberer, E., *et al.*, EMBO, 11:4815 (1992); Ramer, S. W., *et al.*, PNAS, 90:452 (1993). Several mammalian homologs to Ste20 have since been identified, including MST1 (Creasy, C.L., *et al.*, J. Biol. Chem., 271: No. 35, 21049 (1996)), MST2 (Gene, 167:303 (1995)), HPK1 (Kiefer, F., *et al.*, EMBO, Vol. 5, 24:7013 (1996)). Recently, much progress has been made in defining the signal transduction pathways mediating the cellular response to stress. Pombo, C.M., *et al.*, for instance, report the cloning and characterization of a human Ste20-like oxidant stress response kinase, SOK-1. The kinase is positively regulated by phohsphorylation and negatively regulated by its C-terminal non-catalytic region. Reported data suggests SOK-1 transduces signals in response to oxidative and environmental stress. EMBO, Vol. 15, 17:4537 (1996). Moreover, Schinkmann, K., *et al.*, recently reported the cloning and characterization of the human Ste20-like kinase, mst-3. The mst-3 transcript is reported to be ubiquitously expressed. Mst-1 is furthermore reported to be positively regulated by autophosphorylation. J. Biol. Chem., 272(45):28695 (1997). Other stress-activated protein kinase (SAPK), members of the MAPK family, have been shown to be activated *in situ* by inflammatory stimuli, including tumor-necrosis factor (TNF) and interleukin-1. Kyriakis, J.M., *et al.*, Nature, 369:156 (1994); Dérijard, B., *et al.*, Cell, 76:025 (1994); Sánchez, I., *et al.*, Nature, 372:794 (1994). *See also*, Kiefer, F., *et al.*, EMBO, Vol. 5, 24:7013 (1996); Creasy, C.L., *et al.*, J. Biol. Chem., 271: No. 35, 21049 (1996)); Creasy, C.L., *et al.*, Gene, 167:303 (1995)); Manser, E., *et al.*, Nature, 367:40 (1994); Hu, Mickey C.-T., *et al.*, Genes & Development, 10:2251(1996); Katz, P., *et al.*, J. Biol. Chem., (1994)); Pombo, C.M., *et al.*, Nature, 377:750 (1995). Mammalian Ste20-like kinases, including p21-activated protein kinases (PAK) (Manser, E., *et al.*, Nature, 367:40 (1994)) and germinal center kinase (GC kinase) (Katz, P., *et al.*, J. Biol. Chem., (1994)), have been shown to be capable of activating mammalian MAPK cascades. Pombo, C.M., *et al.*, EMBO, Vol. 15, 17:4537 (1996).

Evidence has demonstrated that mitogen-activated protein kinase (MAPK) and stress activated protein kinase (SAPK) signal transduction pathways are responsible for triggering biological effects across a wide variety of pathophysiological conditions including acute and chronic inflammatory disease, auto-immune disorders, rheumatoid arthritis, osteoarthritis, transplant rejection, macrophage regulation, endothelial cell regulation, angiogenesis, peripheral vascular disease, secretion, apoptosis, atherosclerosis, neurogenerative processes, cell senescence, fibroblasts regulation, pathological fibrosis, asthma, allergic response, ARDS, atheroma, osteoarthritis, heart failure, cancer, diabetes, obeisity, cachexia, Alzheimers disease, sepsis, and neurodegeneration as well as conditions manifested by dysfunctional leukocytes and T-lymphocytes. As MAP kinases play a central role in signaling events which mediate cellular response to stress, their inactivation is key to the attenuation of the response. N. J. Holbrook, *et al.*, *Stress-Inducible Cellular Responses*, 273, Feige, U., et al., Eds., Birkhauser Verlag (1996).

Small GTPases

Small GTPases, for example rho-like p21GTPases, e.g., p21 GTPase, Cdc42, and Rac, are known to regulate a broad range of cellular functions by interacting with specific effector components of protein kinase cascades. These P21s act as molecular switches, being active in the GTP-bound form and inactive in the GDP-bound form to which they are converted by the GTPase activity. Signal transduction protein kinases, for example stress activated protein kinases and/or mitogen activated protein kinases, can be regulated as effectors by Ras, Rac and Cdc42. It has become clear that the regulation of protein kinase cascades is a general feature of the biology of each of the small GTPases in the Ras superfamily. Elements for coupling the small GTPases, e.g., Rac and Cdc42, to the stress activated protein kinases are the p21-activated serine/threonine kinases (PAKs). The similarity of PAK to the *Saccharomyces cerevisiae* kinase Ste20, led to experiments showing that Cdc42 regulates Ste20 in this MAPK pathway. This similarity has also led to the demonstration that mammalian Cdc42 and Rac can signal to the nucleus through MAPK pathways. Like Ste20, the PAK family of serine/threonine kinases are each activated directly upon interaction with the GTP-bound form of the small GTPases. PAKs are implicated in the the potent activation of the SAPKs by ligands that signal through heterotrimeric G protein-coupled receptors, a situation analogous to the recruitment of Ste20p by the heptahelical pheromone receptor.

Kyriakis, John M., *et al.*, *Sounding the Alarm: Protein Kinase Cascades Activated by Stress and Inflammation,* JBC, 271:40, 24313 (1996); Lim, Louis, *et al., Regulation of Phosphorylation Pathways by p21 GTPases*, Eur. J. Biochem, 242:171 (1996). *See also*, Benton, Benjamin K. *et al., Cla4p, a Saccaromyces cerevisiae Cdc42p-Activated Kinase Involved in Cytokinesis, Is Activated at Mitosis*, Molecular and Cellular Biology, 17(9):5067 (1997); Bagrodia, Shubha, *et al., Identification of a Mouse* $p21^{Cdc32/Rac}$ *Activated Kinase,* J Biol Chem, 270(39):22731 (1995).

PAK

The JNK pathway has recently been shown to be activated by the Rho family GTPases, Cdc42 and Rac1. Through expression of constitutive hPAK1 kinase in mammalian cells, Brown *et al.* have shown that hPAK1 specifically activates the JNK1 MAP kinase pathway in a manner analogous to the activation of the mating MAP kinase pathway by Ste20 in yeast. Brown, J.L., *et al., Human Ste20 Homologue hPAK1 Links GTPases to the JNK MAP Kinase Pathway,* Current Biology, 6(5):598 (1996). *See also*, Sells, Mary Ann, *et al., Human P21-Activated Kinase (PAK1) Regulates Actin Organization In Mammalian Cells,* Current Biology, 7:202 (1997). Furthermore, human PAK65 (PAK2) has been demonstrated to bind rac1 and CDC42Hs in a GTP-dependent manner and function as an effector molecule for rac1 and CDC42Hs. Martin, George A., *et al. A Novel Serine Kinase Activated by Rac1/CDC42Hs-Dependent Autophosphorylation Is Related to PAK 65 and STE 20,* EMBO, 14(9):1970 (1995); EMBO, 14(17):4835 (1995). *See*, U.S. Patent No. 5,518,911, Human PAK65 [PAK2] issued May 21, 1996; U.S. Patent No. 5,605,825, issued Feb. 25, 1997; U.S. Patent No. 5,698,428, issued December 16, 1997; and U.S. Patent No. 5,698,445 issued December 16, 1997.

Integral members of cellular signaling pathways as targets for therapeutic development, for example, have been the subject to several reviews. *See, e.g.*, Levitzki, A., *Signal-Transduction Therapy: A Novel Approach to Disease Management*, Eur. J. Biochem, 226:1 (1994); Powis G., *The Potential for Molecular Oncology to Define New Drug Targets*, in: New Molecular Targets for Cancer Chemotherapy, Workman, P., Kerr D.J., eds., CRC Press, Boca Raton FL (1994). A novel class of pyridinyl imidazoles, CSAIDS, for instance, have been developed, that inhibit the production of the cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF-α) in monocytes. The drug has been demonstrated to bind

specifically to one protein in monocytes, termed CSBP (CSAID-binding protein), which has been isolated, cloned, and sequenced and demonstrated as a MAPK homolog. Lee, J.C., et al., *Differential Effects of the BicyclicImidazoles on Cytokine Synthesis in Human Monocytes and Endothelial Cells*, Agents Actions, 41:Ć191 (1994); *A Protein Kinase Involved in the Regulation of Inflammatory Cytokine Biosynthesis*, Nature, 372:739 (1994). Moreover, as demonstrated by the identification of rapamycin as a *specific* inhibitor of the activation of p70 S6 kinase and the identification of compounds that inhibit the EGF receptor protein kinase very potently and that block the activation of MAP kinase kinase have demonstrated that specific inhibitors of protein kinases can indeed be developed. Alessi, D., et al., *A Specific Inhibitor of the Activation of MAP Kinase Kinase-1 in vitro and in vivo*, J. Biol. Chem., 279:27489 (1995).

Compounds which are able to modulate the activity of specific signal transduction molecules integral to specific intracellular pathways are expected to have significant potential for the ability to control or attenuate downstream pathophysiological responses. Moreover, in view of the roles for rho-like p21 GTPases in the mediation of disease conditions a need clearly exists for molecules that directly or indirectly selectively modulate the biological and/or pharmacological activities of these proteins. Accordingly, the ability to identify such compounds is of paramount importance.

Summary of the Invention

The present invention is directed to an isolated and purified polynucleotide molecule comprising a nucleic acid sequence which encodes a polypeptide comprising the sequence as depicted in SEQ ID NO:3 or a variant of SEQ ID NO:3 having at least about 80% homology to a member selected from the group consisting essentially of: (SEQ ID NO:3, SEQ ID NO:3 positions 1-413, SEQ ID NO:3 positions 414-653, SEQ ID NO:8, SEQ ID NO:8 positions 414-653, SEQ ID NO:11, SEQ ID NO:11 positions 1-413).

Isolated and purified polynucleotides of the present invention include but are not limited to sequences comprising SEQ ID NO:1 and SEQ ID NO:2.

The current invention is directed to a purified polypeptide comprising the amino acid sequence as depicted in SEQ ID NO:3 or a variant thereof as defined herein.

A preferred embodiment of the invention is an isolated and purified biologically effective antisense polynucleotide molecule comprising an oligomer in the range from about

12 to about 25 nucleotides in length which is complementary to a region within positions 97-178 of SEQ ID NO:1.

The present invention is also directed to an isolated and purified polynucleotide molecule comprising a nucleic acid sequence which encodes a biologically effective dominant negative mutant polypeptide variant of SEQ ID NO:3 which has the ability to modulate the biological activity and/or pharmacological activity of the signal transduction biomolecule of the present invention. A further preferred embodiment of the invention is a biologically effective dominant negative mutant polypeptide variant of SEQ ID NO:3

The invention is further directed to an expression vector for the expression of a signal transduction polypeptide in a recombinant host cell, wherein said vector comprises a nucleic acid sequence which encodes a polypeptide as depicted in SEQ ID NO:3 or a pharmacologically and/or biologically active or biologically effective derivative thereof. The invention is also directed to an expression vector for the expression of a biologically effective antisense polynucleotide molecule comprising a nucleic acid sequence derived from the complement of SEQ ID NO:1 as well; as host cells which harbor each of the said expression vectors.

The instant invention is further directed to methods of identifying compounds that modulate a biological and/or pharmacological activity of a signal transduction molecule, which comprise:

a) combining a candidate compound modulator of signal transduction activity with a polypeptide having the sequence as depicted in SEQ ID NO:3 or a variant thereof, and

(b) measuring an effect of the candidate compound modulator on the biological and/or pharmacological activity of the polypeptide.

The present invention is also directed to compounds identified by means of the aforementioned method, wherein said compound modulates the biological and/or pharmacological activity of a signal transduction molecule.

Additionally, the invention is directed to methods of treatment of a patient in need of such treatment for a condition which is mediated by a signal-transduction molecule, comprising administering: (a) an effective amount of a compound identified by means of the aforementioned method; and/or (b) an effective amount of a polynucleotide which encodes a

biologically effective dominant negative mutant polypeptide comprising the sequence as depicted in SEQ ID NO:3 or a contemplated variant thereof; and/or (c) an effective amount of a biologically effective antisense molecule derived from the complement of SEQ ID NO:1.

The current invention is also drawn toward an antibody specific for a purified polypeptide comprising the amino acid sequence as depicted in SEQ ID NO:3, as well as a diagnostic composition for the identification of a polypeptide sequence comprising the amino acid sequence substantially as shown in SEQ ID NO:3.

The invention is also directed to PCR primers derived from SEQ ID NO:1 as well as to methods of making nucleic acid molecules substantially as shown in SEQ ID NO:1 and SEQ ID NO:2.

Brief Description of the Figures

Figure 1 shows a comparison between the amino acid residue sequence of the human signal transduction serine threonine protein kinase described herein (SEQ ID NO:3) (designated TEN-2), and PAK-1 (SEQ ID NO:4) and PAK-2 (SEQ ID NO:5). Conserved amino acid residues are boxed. Dashes represent gaps introduced to optimize the alignment. Sequences shown in this figure were produced using the Clustal alignment program of DNAStar software (DNAStar Inc., Madison, WI).

Detailed Description of the Invention

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All publications and patents referred to herein are incorporated by reference.

Biological activity as used herein in reference to the signal transduction serine threonine protein kinase of the present invention refers to the ability of the biomolecule to perfom any one or more of the functions including but not limited to the ability to autophosphorylate, to phosphorylate a substrate, to bind ATP, to bind a small rho-like GTPase, to bind p21 GTPase, to bind rac1, to bind Cdc42, to interact with an adaptor protein which contains at least one Src-homolog 3 (SH3) domain, to interact with the adaptor protein Nck.

Pharmacological activity as used herein in reference to the signal transduction serine threonine protein kinase of the present invention refers to bound-GTPase phosphate release activity (the GTPase may be a GTP-bound form, for example, a small rho-like GTPase, p21,

rac1, or Cdc42), activation of p38, SAPK, ERK, or one or more transcription factors, or activation of another terminal kinase or signal transduction molecule, and/or the direct or indirect transcriptional activation of one or more genes; as well as the ability to mediate any one or more of the physiological conditions including but not limited to cell differentiation, proliferation, oncogenic transformation, neoplasia, macrophage regulation, endothelial cell regulation, fibroblast regulation, cytoskeletal structure, metastases, cell aggregation, cell motility, cytokinesis, cancer, angiogenesis, cell senescence, acute and chronic inflammation, auto-immune disorders, arthritis, neurogenerative processes, allergic response, stress response, secretion, apoptosis, cachexia, neurological disorders, peripheral vascular disease, atherosclerosis, heart disease, asthma, atheroma, and the pathogenesis of HIV/AIDS.

Dominant negative mutant as used herein refers to a polypeptide or a nucleic acid coding region sequence which has been changed with regard to at least one position in the sequence, relative to the corresponding wild type native version at a position which changes an amino acid residue position at an active site required for biological and/or pharmacological activity of the native peptide.. Dominant negative mutants of SEQ ID NO:3 contemplated herein include, but are not limited to, polypeptide species which manifest any change with regard to at least one amino acid in the following regions: the CRIB domain at the N-terminal region (positions 12-25 of SEQ ID NO:3); SH3 Binding Domains (PXXP motifs) located in the N-terminal regulatory region (i.e., 163-166, 279-282, 367-370); any change in the residue Asp^{526} in subdomain VI and/or Asp^{544}, and/or Phe^{545}, and/or Gly^{546} from subdomain VII (all of which have been implicated in ATP binding), and/or Asp^{526} (Region VIB); and/or Glu^{571}; or any of the amino acid residues 583 to 588 (subdomain IX).

Biologically effective as used herein in reference to antisense nucleic acid molecules as well as dominant negative mutant nucleic acid coding regions and dominant negative mutant peptides refers to the ability of these molecules to modulate the biological and/or pharmacological activity of the novel signal transduction protein kinase of the present invention, including direct or indirect modulation of transcriptional activation of one or more genes, and/or transcription/translation of nucleic acid coding regions of the novel signal transduction protein kinase of the present invention. *Biologically effective* antisense molecules as well as nucleic acids which encode biologically effective dominant negative

mutant versions of SEQ ID NO:3, or derivatives thereof, are preferred embodiments of the present invention.

As depicted as used herein refers the sequence as well as inherent derivatives thereof, e.g., functional derivative that demonstrate or perform substantially the same biological and/or pharmacological activity in substantially the same way. 'As depicted' is therefore intended to encompass biologically and/or pharmacologically active truncated versions clearly derived from the sequences disclosed and characterized herein (e.g., evidenced domains) as well as chimeric sequences which contain one or more of them.

Variant as used herein refers to sequences substantiallly as shown having changes, e.g., a polypeptide sequence comprising a sequence which differs from the sequence referred to by at least one amino acid substitution, addition, or deletion, preferrably a conservative amino acid substitution, that demonstrate or perform substantially the same biological and/or pharmacological activity in substantially the same way, as well as truncated versions of these variants. However, variant as used herein is intended to encompass all contemplated *biologically effective dominant negative mutants*, several species of which are set forth herein.

The term modulation is used herein to refer to the capacity to either enhance or inhibit a function of a biological molecule including, but not limited to, a biological and/or pharmacological activity of a signal transduction molecule, or to the capacity to either enhance or inhibit a functional property of a nucleic acid coding region. Modulate physiology as used herein refers to the biophysiological regulation of cells and/or tissue and the treatment of pathophysiological disorders related thereto.

Direct administration as used herein refers to the direct administration of nucleic acid molecules, peptides, or compounds as well as contemplated derivatives/variants of the present invention. Direct administration includes but is not limited to *ex vivo* as well as *in vivo* gene therapy techniques.

Purified as used herein refers to molecules, either nucleic acid or amino acid sequences, that are removed from their natural environment and isolated or separated from at least one other component with which they are naturally associated.

Expression vector as used herein refers to nucleic acid vector constructions to direct the transcription of nucleic acid regions in host cells. Expression vectors include but are not limited to plasmids, retroviral vectors, viral and synthetic vectors.

Transformed host cells as used herein refer to cells which harbor one or more nucleic acids of the present invention.

Signal Transduction Kinases

The protein kinases are a large family of enzymes. Conserved structural motifs provide clear indications as to how the kinases transfer the γ-phosphate of a purine nucleotide triphosphate to the hydroxyl groups of their protein substrates. The kinase domains that define protein kinases contain 12 conserved subdomains (I-XII) that fold into a common catalytic core structure, as revealed by the 3-dimensional structures of several enzymes. The central core of the catalytic domain, the region with greatest frequency of highly conserved residues, consists of subdomains VI through IX. The most striking indicator of amino acid specificity is found in subdomain VI, the consensus in this region is a strong indicator of serine/threonine specificity. *See, e.g.*, Hanks, S.K., *et al.*, *The Protein Kinase Family: Conserved Features and Deduced Phylogeny of the Catalytic Domains*, Science, 241:42 (1988); Hanks, S.K., *et al.*, *The Eukaryotic Protein Kinase Superfamily: Kinase (Catalytic) Domain Structure and Classification*, FASEB , Ser. Rev., 9:576 (1995).

The study of isozymes has grown into one of fundamental significance in the investigation of the molecular basis of cellular differentiation and morphogenesis. Isozymes represent a type of regulation accomplished through the participation of multiple forms of a given enzyme or enzyme subunits that occur within an organism, in different cell types, or even within a single cell. In many instances all forms of the particular enzyme catalyze the same overall reaction, e.g., phosphorylation, but differ in their dependence on substrate, substrate concentration, activators, cofactors, and cellular conditions. The relative proportions of isozymes in particular tissues is particularly important in the diagnosis of disease. Isozymes are now known for a great many different enzymes. Many allosteric enzymes occur as two or more isozymes that vary in sensitivity to their allosteric modulators. Different isozymes have accordingly evolved as mechanistic response to biochemical conditions - their presence therefore is a reliable factor in the indication of precise normal physiological or, in contrast, pathophysiological conditions.

Rho-like proteins, p21 proteins, like other GTPases, cycle between an active GTP-bound form and an inactive GDP-bound state. The p21 GTPases are known to be integral components of signal transduction mechanisms leading to regulation of cell proliferation.

Many pathological conditions result from aberrant control of cell proliferation or differentiation. Neoplasia, for example, is characterized by a clonally derived cell population which has a diminished capacity for responding to normal cell proliferation control signals. Oncogenic transformation of cells leads to a number of changes in cellular metabolism, physiology, and morphology. One characteristic alteration of oncogenically transformed cells is a loss of responsiveness to constraints on cell proliferation and differentiation normally imposed by expression of cell growth regulatory genes.

PAK Kinases

PAK (p21, Cdc42, Rac-activated kinase) defines a growing family of mammalian kinases that are related to yeast Ste20 and are activated through binding to p21, Cdc42, and Rac1. The p21-activated serine threonine protein kinases (PAKs) are regulatory enzymes that have roles in diverse phenomena such as cellular morphogenesis and proliferation, stress response, and the pathogensis of HIV/AIDS. PAKs were initially discovered as binding partners for small (21 kDa) GTPases and recent evidence has shown that members of the PAK family may be effectors for related GTPases. The defining member of the p21-activated protein kinase (PAK) family was isolated as a Rac1-binding protein from rat brain. Manser, E., *et al.*, Nature, 367:40 (1994). This protein binds preferentially to the activated (GTP-bound) form of Rac1 and the related protein Cdc42 but not to other GTPases, and this association causes the kinase activity of PAK to increase markedly. These properties make PAKs attractive candidates for effectors for Rac and Cdc42, members of the Rho subfamily of p21 GTPases. Sells, M.A., *et al., Emerging from the PAK: The p21-Activated Protein Kinase Family,* Trends in Cell Biology, Vol 7:162 (1997).

PAK1

PAK and Ste20 kinases play key parts in linking extracellular signals from membrane components, such as receptor-associated G proteins and Rho-related GTPases, to nuclear responses, such as transcriptional activation. The JNK pathway has recently been shown to be activated by the Rho family GTPases, Cdc42 and Rac1. Brown *et al.* have demonstrated that the human PAK homolog, hPAK1, acts as a GTPase effector which links the Rho-related GTPases to the JNK MAP kinase pathway. Brown, Jeffrey L., *et al., Human Ste20 Homologue hPAK1 Links GTPases to the JNK MAP Kinase Pathway,* Current Biology, 6(5):598 (1996). Moreover, PAK1 mutant displays enhanced binding to the adaptor protein

Nck, which contains three Src-homolog 3 (SH3) domains. Sells, M.A., *et al., Human P21-Activated Kinase (PAK1) Regulates Actin Organization In Mammalian Cells,* Current Biology, 7:202 (1997). All known mammalian PAKs, as well as *Drosophila* and *Caenorhabditis elegans* PAK1, have a highly conserved, proline-rich motif which directs interactions with proteins that contain a Src-homology 3 (SH3) domain, such as Nck. Galisteo, M.L., *et al., The Adaptor Protein Nck Links Tyrosine Kinases with the Serine-Threonine Kinase PAK1,* J. Biol. Chem, 271:20997 (1996); Bokoch, G.M., *et al., Interaction Of Nck Adapter Protein With P21-Activated Kinase* (PAK1), J. Biol. Chem., 271:25746 (1996); Zhang, S., *et al., Rho Family GTPases Regulate P38 Mitogen-Activated Protein Kinase through the Downstream Mediator Pak1*, J Biol Chem, 270:23934 (1995). Sells, *et al.* and others have shown that mammalian PAKs mediate activation of stress-activated protein kinase cascades by Rac1/Cdc42. Sells, M.A., *et al.,* Current Biology, 7:202 (1997).

Human PAK65 (PAK2) can bind rac1 and CDC42Hs in a GTP-dependent manner and functions as an effector molecule for rac1 and CDC42Hs. Martin, George A., *et al. A Novel Serine Kinase Activated by Rac1/CDC42Hs-Dependent Autophosphorylation Is Related to PAK 65 and STE 20,* EMBO, 14(9):1970 (1995); EMBO, 14(17):4835 (1995). Upon binding various GTPases hPAK65 autophosphorylates, thereby increasing its catalytic activity towards exogenous substrates. *See*, U.S. Patent No. 5,518,911, Human PAK65 [PAK2] issued May 21, 1996; U.S. Patent No. 5,605,825, issued Feb. 25, 1997; U.S. Patent No. 5,698,428, issued December 16, 1997; and U.S. Patent No. 5,698,445 issued December 16, 1997. Materials and methods described in these issued patents are herein incorporated by reference

CRIB (Cdc42/Rac1 interactive binding), is highly conserved in all members of the PAK family and confers on these proteins the ability to bind to activated Cdc42 and/or Rac1. Burbelo *et al.* have localized the Cdc42 binding site to a minimal conserved region of 16 amino acids. Burbelo, Peter D. *et al. A Conserved Binding Motif Defines Numerous Candidate Target Proteins for Both Cdc42 and Rac GTPases,* The Journal of Biological Chemistry 270:49, 29071 (1995).

Other notable features present in mammalian PAKs are a highly conserved proline-rich sequence at the extreme N-terminus and a stretch of acidic residues. The N-terminal proline-rich region has been shown to mediate binding of PAK to the second Src-homology 3 (**SH3**) domain of the adaptor protein Nck and quite possibly to other SH3-containing proteins.

Galisteo, M.L., *et al.*, J. Biol. Chem., 271:20997 (1996); Bokoch, G.M., *et al.*, J. Biol. Chem. 271:25746 (1996).

A trio of reports has recently documented that the human immunodeficiency virus (HIV) encoded protein Nef associates with and activates one or more PAK-like kinases. *See*, Cullen, B.R., *et al.*, Curr. Biol. 6:1557 (1996); Sells, Mary Ann, *et al.*, *Emerging from the PAK: The p21-Activated Protein Kinase Family*, Trends in Cell Biology, Vol 7:162 (1997).

SEQ ID NO:4 is the 545 amino acid residue sequence of human PAK1 as described by Sells, M., Jonathan Chernoff, *et al.*, Fox Chase Cancer Center, Philadelphia, PA (cDNA locus HSU24152, 2318 bp mRNA, GENBANK accession U24152. *See, also*, Brown J.L., *et al.*, *Human Ste20 Homologue hPAK1 links GTPases to the JNK MAP Kinase Pathway*, Curr. Biol. 6:598 (1996) (submitted to Genbank under accession U51120).

SEQ ID NO:5 is the 525 amino acid residue sequence of PAK2 (PAK65) as described by Sells, M., Jonathan Chernoff, *et al.*, Fox Chase Cancer Center, Philadelphia, PA (cDNA locus HSU24153, 2019 bp mRNA, GENBANK accession U24153. *See, also*, Martin, G.A., *et al.*, EMBO, 14:1970 (1995); and, EMBO, 14:4385 (1995).

<u>Novel Human Signal Transduction Serine Threonine Protein Kinase (GTPase Effector)</u>

Protein kinases which have closely related catalytic domains, and thus define a family, represent products of genes that have undergone relatively recent evolutionary divergence. Clustering appears to be of predictive value in the determination of the properties and function of novel protein kinases. Accordingly, members of a given family tend also to share related functions. This is manifest by similarities in overall structural topology, mode of regulation, and substrate specificity. *See, generally*, Hardie, D. G,. *et al.*, The Protein Kinase Facts Book, Academic Press, London (1995). *See* FIG.1.

A human signal transduction serine/threonine protein kinase molecule as well as example nucleic acid sequences and example methods of making and using them are herein described. A cDNA sequence is provided SEQ ID NO:1 which comprises the structural coding region of the native human signal transduction kinase SEQ ID NO:2. SEQ ID NO:1 positions 1-128 represent the 5' leader sequence or UTR; the structural coding region for SEQ ID NO:3 is represented by positions 129-2174 (otherwise SEQ ID NO:2); positions 162-203 encode the cdc42/Rac interactive binding domain of SEQ ID NO:3; the 3'UTR is represented by positions 2175-3600. SEQ ID NO:1 positions 123-128 further represent a

'Kozak sequence'; positions 1368-2087 further demonstrate the coding region for the the kinase catalytic domain of SEQ ID NO:3 discussed in more detail *infra*.

SEQ ID NO:2 is the 2046bp structural region, ATG to TGA, of the cDNA nucleic acid sequence which encodes the human signal transduction serine threonine protein kinase.

SEQ ID NO:3 is the 691 amino acid residue sequence of the human signal transduction serine threonine protein kinase of the present invention. The novel kinase appears to belong to the Ste-20 kinase family of MAP Kinases; however, sequence similarity suggests that this kinase is a new member of the p21 activated kinases (PAK's). The initial stimulant leading to the activation of the signal transduction serine threonine protein kinase therefore may be stress factors, reactive oxygen species, mitogens/growth factors, and the like. Activation of SEQ ID NO:3 is therefore expected to result in the stimulation of the MAP kinase cascade the result of which may be the activation of p38, SAPK, ERK or other terminal kinase which in turn can activate pharmacologically important transcription factors, indicating that SEQ ID NO:3 may be important in cell cycle and proliferation. Furthermore, the PAK's have also been implicated in the restructuring of cytoskeletal proteins (e.g. actin, myosin and related proteins). This indicates that the SEQ ID NO:3 kinase may be important in cell morphology processes (e.g. angiogenesis, morphogenesis, proliferation, and similar physiological manifestations).

CRIB

The CRIB (Cdc42/Rac Interactive Binding region) or PBD (p21 Binding Domain) GTPase binding domain is located at the N-terminal region of SEQ ID NO:3, i.e., amino acid positions 12-25. *See*, Burbelo, P., *et al.*, JBC, 270:29071 (1995). Binding of the novel protein kinase SEQ ID NO:3 to a small GTPase is expected to result in autophosphorylation of the kinase resulting in increased activity toward exogenous substates. SEQ ID NO:3 is expected to be an effector for any number of small GTPase (including but not limited to Rac, cdc42, p21) mediated events. Contemplated GTPases include GTP-bound forms as well as other forms.

SH3 Binding Domain

It is well known that SH3 binding domains are capable of binding to SH3 domains of other proteins thereby affording important protein:protien interaction communications between biomolecules. The PAK kinases contain multiple SH3 binding domains and have

been shown to interact with adapter molecules (e.g. Nck). Several important SH3 binding domains are located in the N-terminal regulatory region of the novel GTPase effector kinase, SEQ ID NO:3. Based upon the integral SH3 binding domains, the signal transduction protein kinase SEQ ID NO:3 is expected interact with an *adaptor molecules*, including but not limited to Nck, for example. The interaction of the SEQ ID NO:3 protein kinase with one or more adapter molecules is expected to couple the kinase to receptor tyrosine kinases, plasma membrane proximal effector molecules, and/or membrane distal effector molecules in at least one pharmacologically significant signal transduction cascade.

Catalytic Domain

A serine/threonine kinase catalytic region is present in the C-terminal region of the subject GTPase effector kinase and is represented by positions 414-658 of SEQ ID NO:3. The catalytic region contains all 12 conserved ser/thr kinase domains. Hanks, S., Hunter, T., FASEB, 9:576 (1995). This domain is responsible for the phosphotransferase activity. Amino acid residues 414 to 421 of SEQ ID NO:3, corresponds to the consensus found in protein kinase subdomain I. Subdomain II is involved in the phosphotransfer reaction and identified by an invariant lysine in the tripeptide sequence AxK. This sequence is found in SEQ ID NO:3 residues 434 to 436 (AVK) and is a clear candidate for dominant negative mutations contemplated herein. Subdomains VI through IX form the central core of catalytic activity and is characterized in SEQ ID NO:3 by a large number of highly conserved residues. SEQ ID NO:3 includes the invariant or nearly invariant residues Asp^{526} in subdomain VI and Asp^{544}, Phe^{545}, and Gly^{546} in subdomain VII; all of which have been implicated in ATP binding. Each of these also represent dominant negative mutation sites contemplated herein. Region VIB contains the consensus sequence which includes the invariant Asp^{526} (which is the subject of some example dominant negative mutant versions described herein). The signal transduction serine threonine protein kinase contains the conserved DFG of subdomain VII (SEQ ID NO:3 positions 563-573). The Asp functions to orient the γ-phosphate of the ATP for transfer; this position also represents a dominant negative mutation site. Subdomain VIII of the SEQ ID NO:3 kinase contains the highly conserved APE sequence, with the Glu corresponding to the invariant Glu^{571} (a preferred dominant negative mutation site). This subdomain is believed to play a critical role in the recognition of substrate binding. Moreover, many kinases are known to be activated by phosphorylation of residues in

subdomain VIII. The consensus sequence of subdomain IX is represented by SEQ ID NO:3 amino acid residues 583-588. This region forms a large α-helix and the initial Asp of the consensus sequence serves to stabilize the catalytic loop by hydrogen bonding.

The chromosomal gene corresponding to the signal transduction serine threonine protein kinase described herein is predominately transcribed in skeletal muscle, heart, pancreas, prostate, placenta, as well as lower levels in other tissues.

Significant evidence has been provided that kinase activation pathways are responsible for biological effects across a wide variety of disease areas. As MAP and PAK kinases play a central role in signaling events which mediate cellular responses, compounds which modulate or inactivate specific integral signal transduction molecules, i.e., the GTPase effector kinase molecule described herein, SEQ ID NO:3, or nucleic acid sequences coding therefor, e.g. SEQ ID NO:1 and/or SEQ ID NO:2, have significant potential for the ablity attenuate pathophysiological responses. The ability to screen for antagonists and/or agonists which modulate the biological and/or pharmacological activity of the native human serine/threonine signal transduction kinase molecule as described herein is significantly valuable toward the identification and development of therapeutic agents. Moreover, diagnostic applications are readily apparent for the detection of pathophysiological conditions manifested by abnormal levels of molecules such as SEQ ID NO:2 and/or SEQ ID NO:3 by means of PCR sequence amplification and subsequent detection and/or antibody based assays, e.g., ELISA-based assays, which are well-known to those skilled in the art and readily performed provided the information disclosed herein.

The present invention relates to nucleic acid sequences and amino acid sequences of the novel human signal transduction kinase and variants thereof and to the use of these sequences to identify compounds that modulate the biological and/or pharmacological activity of a signal transduction molecule.

Polynucleotide sequences which encode the human signal-transduction molecule as depicted in SEQ ID NO:3 and variants thereof contemplated herein are particularly preferred embodiment of the present invention.. *Biologically effective* antisense molecules and nucleic acids which encode biologically effective dominant negative mutant versions of SEQ ID NO:3, or derivatives thereof, as well as dominant negative mutant versions of SEQ ID NO:3, and derivatives thereof, examples of each of which are described *infra*, are preferred

embodiments of the present invention and are intended to fall within the scope of the claims appended hereto.

The present invention also provides a method of treatment for a patient in need of such treatment, *videlicet* for a patient who suffers a pathological condition mediated by the SEQ ID NO:3 signal transduction kinase or another signal transduction molecule or a downstream transcriptional activator, comprising administering an effective amount of a biologically effective antisense nucleic acid molecule derived from SEQ ID NO:1 or SEQ ID NO:2; or administering an effective amount of a nucleic acid which encodes a biologically effective dominant negative mutant version of the signal transduction kinase; or administering a compound that modulates the biological and/or pharmacological activity of SEQ ID NO:3 which was identified by a method described herein.

The present invention relates to nucleic acid sequences (e.g., SEQ ID NO:1 and SEQ ID NO:2) and amino acid sequences (e.g., SEQ ID NO:3, SEQ ID NO:8, and SEQ ID NO:11) of the novel human signal transduction kinase as well as inherent derivatives thereof, e.g., functional derivative that demonstrate or perform substantially the same biological and/or pharmacological activity in substantially the same way. The invention is also intended to encompass biologically and/or pharmacologically active truncated versions clearly derived from the sequences disclosed and characterized herein (e.g., evidenced domains described *infra*) as well as chimeric sequences which contain one or more of them.

Variants

The present invention relates to variants of nucleic acid sequences sequences (e.g., SEQ ID NO:1 and SEQ ID NO:2) and amino acid sequences (e.g., SEQ ID NO:3) substantially as shown, which have changes, e.g., a polypeptide sequence comprising a sequence which differs from the sequence referred to by at least one amino acid substitution, preferrably a conservative amino acid substitution, that demonstrate or perform substantially the same biological and/or pharmacological activity in substantially the same way, as well as molecules which comprise truncated versions of these variants. However, variant as used herein is intended to encompass all contemplated *biologically effective dominant negative mutants*, several species of which are set forth herein.

A preferred variant, as depicted in SEQ ID NO:3 for instance, is one having at least 80% amino acid sequence homology (identity) to SEQ ID NO:3; a more preferred variant is

one having at least 90% amino acid sequence homology; and a most preferred variant is one having at least 95% amino acid sequence homology to the kinase molecule amino acid sequence as depicted in SEQ ID NO:3 or a biologically and/or pharmacologically active substantial fragment thereof. Variants within the scope of this invention also include biologically-effective dominant negative mutants of these contemplated embodiments

A variant of the SEQ ID NO:3 human kinase molecule of the present invention may have an amino acid sequence that is different by one or more amino acid substitutions. Embodiments which comprise amino acid deletions and/or additions are also contemplated. The variant may have conservative changes (amino acid similarity), wherein a substituted amine acid has similar structural or chemical properties, for example, the replacement of leucine with isoleucine. A variant may have nonconservative changes, e.g., replacement of a glycine with a tryptophan. Embodiments within the intended scope of the invention also include SEQ ID NO:3 having one or more amino acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without abolishing biological or proposed pharmacological activity may be reasonably inferred in view of this disclosure and may be further be found using computer programs well known in the art, for example, DNAStar software.

Amino acid substitutions of SEQ ID NO:3 may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as a biological and/or pharmacological activity of the native molecule is retained. However, amino acid substitutions are important to construct contemplated *biologically effective dominant negative mutants*, several species of which are set forth herein.

Negatively charged amino acids, for example, include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine phenylalanine, and tyrosine. However, in the construction of biologically effective dominant negative mutants at least one amino acid residue position at an active site required for biological and/or pharmacological activity in the native peptide is changed to produce an agent or entity having reduced activity or which is devoid of detectable native wild type activity.

Suitable substitutions of amino acids include the use of a chemically derivatized residue in place of a non-derivatized residue. D-isomers as well as other known derivatives may also be substituted for the naturally occurring amino acids. *See, e.g.,* U.S. Patent No. 5,652,369, *Amino Acid Derivatives*, issued July 29, 1997. Example substitutions are set forth in Table 1 as follows:

Table 1

Original residue	Example conservative substitutions
Ala (A)	Gly; Ser; Val; Leu; Ile; Pro
Arg (R)	Lys; His; Gln; Asn
Asn (N)	Gln; His; Lys; Arg
Asp (D)	Glu
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln; Arg; Lys
Ile (I)	Leu; Val; Met; Ala; Phe
Leu (L)	Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; His; Asn
Met (M)	Leu; Tyr; Ile; Phe
Phe (F)	Met; Leu; Tyr; Val; Ile; Ala
Pro (P)	Ala; Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala

"Homology" is a measure of the *identity* of nucleotide sequences or amino acid sequences. In order to characterize the homology, subject sequences are aligned so that the

highest order homology (match) is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. Computer program methods to determine identity between two sequences, for example, include DNAStar software (DNAStar Inc., Madison, WI); the GCG program package (Devereux, J., *et al.*, Nucleic Acids Research (1984) 12(1):387); BLASTP, BLASTN, FASTA (Atschul, S. F. *et al.*, J Molec Biol (1990) 215:403). Homology (identity) as defined herein is determined conventionally using the well known computer program, BESTFIT (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, about 80% homologous to a reference sequence, according to the present invention, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence or amino acid sequence and that gaps in homology of up to about 20% of the total number of nucleotides in the reference sequence are allowed. Eighty percent of homology is therefore determined, for example, using the BESTFIT program with parameters set such that the percentage of identity is calculated over the full length of the reference sequence, e.g., SEQ ID NO:3, and gaps of up to 20% of the total number of amino acids in the reference sequence are allowed, and wherein up to 20% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 20% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. Percent homologies are likewise determined, for example, to identify preferred species, within the scope of the claims appended hereto, which reside within the range of about 80 percent to 100 percent homology to SEQ ID NO:3 as well as biologically and/or pharmacologically active functional derivatives thereof and biologically effective dominant negative mutants contemplated herein..

Percentage *similarity* (conservative substitutions) between two polypeptides may also be scored by comparing the amino acid sequences of the two polypeptides by using programs well known in the art, including the BESTFIT program, by employing default settings for determining similarity.

The present invention relates, in part, to the inclusion of the polynucleotide encoding the novel human signal-transduction kinase molecule in an expression vector which can be

used to transform host cells or organisms. Such transgenic hosts are useful for the production of the protein kinase as well as valuable variations thereof contemplated herein.

The nucleic acid sequence also provides for the design of antisense molecules, example embodiments of which are provided herein, which are useful in downregulating, diminishing, or eliminating expression, e.g., transcription and/or translation of SEQ ID NO:2 in cells.

The human signal-transduction kinase molecule of the present invention is used in screening assays to identify antagonists or inhibitors which bind the novel protein kinase GTPase effector, emulate its substrate, or otherwise inactivate the biomolecule or compete biologically, e.g., competitive interaction or competitive binding inhibition, with the native SEQ ID NO:3 biomolecule. The novel protein kinase GTPase effector can also be used in screening assays to identify agonists which agonize or mimic the biological and/or pharmacological activity, induce the production of or prolong the biological halflife of the molecule *in vivo* or *in vitro*.

The invention also relates to pharmaceutical compositions which comprise molecules as depicted in SEQ ID NO:2 or SEQ ID NO:3 or variants of these molecules as defined herein for the treatment of pathological disorders related to or mediated by the human signal transduction serine threonine protein kinase of the present invention.

Example Embodiments and Dominant Negative Mutants

A purified polynucleotide is preferred which comprises a nucleic acid sequence which encodes a polypeptide comprising the sequence as depicted in SEQ ID NO:3 or a variant of SEQ ID NO:3, including but not limited to, SEQ ID NO:3 positions 1-413, SEQ ID NO:3 positions 414-653, SEQ ID NO:8, SEQ ID NO:8 positions 414-653, SEQ ID NO:11, and SEQ ID NO:11 positions 1-413.

The human signal transduction serine threonine protein kinase (SEQ ID NO:3) coding region, SEQ ID NO:2, can be obtained from existing cDNA (e.g., ORIGENE (Rockville, MD) Placenta cDNA library or other suitable cDNA source, for example, by PCR ampflication using primers, e.g. SEQ ID NO:6 and SEQ ID NO:7, as described in Example VIII. SEQ ID NO:2, for example, (as well as variations thereof) may be inserted into expression vectors, with or without fusion protein "tags", for expression of a protein, e.g., SEQ ID NO:3, which can be purified and screened for biological and/or pharmacological activity, including signal

transduction activity, and substrate activation. Prokaryotic expression vectors may be used including the likes of, for example, pGEX (GST fusion), pET (+/- His_6 or T7 tag), as well as eukaryotic expression vectors including the likes of, for example, pcDNA3.1His, pIRES-EGFP, pcDNA3, and pEBVHis. Recombinant proteins, derived from SEQ ID NO:3, are provided for use in screening assays for the identification of compounds which may modulate the biological and/or pharmacological activity of signal transduction serine threonine protein kinases.

An example dominant negative mutant of SEQ ID NO:3 (lysine at position 436 changed to arginine) is set forth in SEQ ID NO:8. SEQ ID NO:8 is an embodiment predicted to be catalytically inactive due the substitution of the lysine in the kinase domain II. Lysine 436 of SEQ ID NO:3 is known to be an important residue in ATP binding and therefore the biological activity of this type of kinase. Example Primers (SEQ ID NO:9 and SEQ ID NO:10) are provided as tools to synthesize the SEQ ID NO:8 dominant negative mutant coding region. *See*, Example X.

A further example dominant negative mutant (SEQ ID NO:11) of the subject signal transduction serine threonine protein kinase includes the mutation of lysine 436 to arginine as well as histidine 20 to leucine and histidine 23 to leucine. The SEQ ID NO:11 embodiment is expected to lack the biological ability to bind to family members of small rho-like GTPases, including, but not limited to, the likes of p21, rac1, Cdc42. Example Primers (SEQ ID NO:12 and SEQ ID NO:13) are provided as tools to synthesize the SEQ ID NO:11 dominant negative mutant coding region. *See*, Example XI.

An example pharmacologically significant embodiment which comprises the regulatory domain of the signal transduction serine threonine protein kinase, e.g., SEQ ID NO:3 positions 1-413, is a preferred embodiment of the present invention. A coding region for such an example embodiment may be synthesized by means of PCR amplification from, for example, the SEQ ID NO:1 cDNA using the following 2 primers: sense 5'-GCCCCGAATTCGGCACCATGTTCCGCAAG (SEQ ID NO:14); and, antisense 5'-CTCGTGGCGGCCGCTCACCGGGGGTCACCCTGGTCCAC (SEQ ID NO:15). A polypeptide comprising the sequence SEQ ID NO:11 positions 1-413, as well as a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide comprising

such an amino acid sequence, are further example pharmacologically significant embodiments of the current invention.

A further example pharmacologically significant embodiment which comprises the catalytic region of the signal transduction serine threonine protein kinase, e.g., SEQ ID NO:3 positions 414-653, is another example of a preferred embodiment of the invention. In this embodiment the serine/threonine kinase catalytic region is present and represented by the C-terminal region of SEQ ID NO:3. A coding region for such an example embodiment may be synthesized by means of PCR amplification from, for example, the SEQ ID NO:1 cDNA using the following 2 primers: sense 5'-GCCCCGAATTCATGCTGCTGCTGGACAGCTACGTG (SEQ ID NO:16); antisense 5'-CTCGTGGCGGCCGCTCAGCAGGTGGAGGTCTG (SEQ ID NO:17). A polypeptide comprising the sequence SEQ ID NO:8 positions 414-653, as well as a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide comprising such an amino acid sequence are further example pharmacologically significant embodiments of the current invention.

Antisense Molecules

Various nucleic acid sequences complementary to SEQ ID NO:1 and SEQ ID NO:2 provided herein may be used in another embodiment of the invention to modulate the expression of a signal-transduction biomolecule by affecting the transcription and/or translation of the subject sequences in cells. Pharmacological activity of the endogenous gene may be modulated by affecting the transcription and/or translation, for example, of the endogenous gene by use or administration of anti-sense constructs to produce anti-sense transcripts or by direct delivery of anti-sense oligomers. Antisense constructs and oligomers may each be used as embodiments of the present invention and each are related to therapeutic method embodiments practiced *via* direct administration as defined herein. Translation is most effectively inhibited by blocking the mRNA at a site at or near the initiation codon. Thus, oligonucleotides complementary to the 5' -terminal region of the GTPase effector kinase mRNA transcript are preferred.

Antisense molecules which comprise oligomers in the range from about 12 to about 25 nucleotides which are complementary the regions of SEQ ID NO:1 and/or 5' region pSEQ ID NO:2 which are proximal to, or include, the translational start codon, or a portion thereof, are

preferred embodiments of the invention. Antisense molecules comprising oligomers from about 12 to about 25 nucleotides in length which are complementary to a region within the SEQ ID NO:1 positions 97-178 are particularly preferred embodiments. Oligonucleotides which comprise sequences complementary to the following positions of SEQ ID NO:1 are example embodiments of the invention: SEQ ID NO:1 positions 97-109; 100-112; 116-128; 123-135; 124-136; 125-137; 126-138; 127-139; 128-140; 129-141; 130-142; 131-143; 132-144; 133-145; 134-146; 135-147; 136-148; 137-149; 138-150; 139- 151; 140-152; 141-153; 142-154; 143-155; 144-156; 145-157; 146-158; 147-159; 148-160; 149-161; 150-162; 151-163; 152-164; 153-165; 154-166; 155-167; 156-168; 157-169; 158-170; and 166-178. Oligonucleotides which comprise sequences complementary to and hybridizable to the recited area of the novel human signal-transduction kinase mRNA are contemplated for therapeutic use. Moreover, U.S. Patent No. 5,639,595, *Identification of Novel Drugs and Reagents*, issued Jun. 17, 1997, wherein methods of identifying oligonucleotide sequences that display *in vivo* activity are thoroughly described, is herein incorporated by reference.

Nucleotide sequences that are complementary to the signal-transduction molecule encoding nucleic acid sequence can be synthesized for antisense therapy. These antisense molecules may be DNA, stable derivatives of DNA such as phosphorothioates or methylphosphonates, RNA, stable derivatives of RNA such as 2'-O-alkylRNA, or other oligonucleotide mimetics. U.S. Patent No. 5,652,355, *Hybrid Oligonucleotide Phosphorothioates*, issued July 29, 1997, and U.S. Patent No. 5,652,356, *Inverted Chimeric and Hybrid Oligonucleotides*, issued July 29, 1997, which describe the synthesis and effect of physiologically-stable antisense molecules, are incorporated by reference. Signal-transduction GTPase effector kinase antisense molecules may be introduced into cells by microinjection, liposome encapsulation or by expression from vectors harboring the antisense sequence. Antisense therapy may be particularly useful for the treatment of diseases where it is beneficial to reduce the signal-transduction biological and/or pharmacological activity.

Gene Therapy

Embodiments of signal-transduction nucleic acids or dominant negative mutant versions thereof as well as antisense embodiments described herein may be administered to a subject *via* gene therapy to modulate, i.e., boost or attenuate the corresponding biological and/or pharmacological activity or gene expression of an endogenous GTPase effector kinase.

Nucleic acid sequences of the present invention may be delivered *ex vivo* or *in vivo* to the cells of target organs in a tissue-specific manner. The human signal-transduction kinase polypeptide coding region can be ligated into viral vectors which mediate transfer of the kinase polypeptide DNA by infection of recipient host cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, polio virus and the like. *See, e.g.*, U.S. Patent No. 5,624,820, *Episomal Expression Vector for Human Gene Therapy*, issued April 29, 1997. GENOVO Corporation, for instance, Sharon Hill, PA, at the date of this application, have a readily commercially available expression vector portfolio which comprise an assortment of vectors complete with well-established methods which consistently demonstrate tissue-specific expression. The GENOVO Corporation is an example source for vectors and methods to practice gene-therapy methods of the present invention. Nucleic acid coding regions of the present invention are incorporated into effective expression vectors, which are directly administered or introduced into somatic cells for gene therapy (a nucleic acid fragment comprising a coding region, preferably mRNA transcripts, may also be administered directly or introduced into somatic cells). *See, e.g.*, U.S. Patent No. 5,589,466, issued Dec. 31, 1996. Such nucleic acids and vectors may remain episomal or may be incorporated into the host chromosomal DNA as a provirus or portion thereof that includes the gene fusion and appropriate eukaryotic transcription and translation signals, i.e, an effectively positioned RNA polymerase promoter 5' to the transcriptional start site and ATG translation initiation codon of the gene fusion as well as termination codon(s) and transcript polyadenylation signals effectively positioned 3' to the coding region. Alternatively, the human signal-transduction kinase polypeptide DNA can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted DNA transfer using ligand-DNA conjugates or adenovirus-ligand-DNA conjugates, lipofection membrane fusion or direct microinjection. These procedures and variations thereof are suitable for *ex vivo*, as well as *in vivo* human signal-transduction kinase polypeptide gene therapy according to established methods in this art.

Generally Acceptable Vectors

In accordance with the present invention, polynucleotide sequences which encode the GTPase effector kinase, fragments of the polypeptide, fusion proteins or functional equivalents thereof may be used in recombinant DNA molecules that direct the expression of

the respective molecule in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used to clone and express the signal transduction serine threonine protein kinase, as well as variations thereto and dominant negative mutants thereof. As will be understood by those of skill in the art, it may be advantageous to produce nucleotide sequences possessing non-naturally occurring codons.

Cloned signal transduction kinase cDNA obtained through the methods described herein may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce the GTPase effector kinase. Techniques for such manipulations are fully described in Sambrook, J., *et al.*, Molecular Cloning Second Edition, Cold Spring Harbor Press (1990), and are well known in the art.

Expression vectors are described herein as nucleic acid sequences for the transcription of embodiments of the present invention. Such vectors can be used to express nucleic acid sequences in a variety of hosts such as bacteria, bluegreen algae, plant cells, insect cells, fungal cells, human, and animal cells. Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast, or bacteria-animal cells, or bacteria-fungal cells, or bacteria-invertebrate cells.

A variety of mammalian expression vectors may be used to express the recombinant human GTPase effector kinase molecule as well as variants contemplated herein. Commercially available mammalian expression vectors which are suitable for recombinant expression, include but are not limited to, pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and lZD35 (ATCC 37565), pLXIN and pSIR (CLONTECH), pIRES-EGFP (CLONTECH). INVITROGEN corporation provides a wide variety of commercially available mammalian expression vector/systems which can be effectively used with the present invention. INVITROGEN, Carlsbad, CA. *See, also*, PHARMINGEN products, vectors and systems, San Diego, CA.

Baculoviral expression systems may also be used with the present invention to produce high yields of biologically active kinase. Vectors such as the CLONETECH, BacPak™

Baculovirus expression system and protocols are preferred which are commercially available. CLONTECH, Palo Alto, CA. Miller, L.K., *et al.*, Curr. Op. Genet. Dev. 3:97 (1993); O'Reilly, D.R., *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual*, 127. Vectors such as the INVITROGEN, MaxBac™ Baculovirus expression system, insect cells, and protocols are also preferred which are commercially available. INVITROGEN, Carlsbad, CA.

Example Host Cells

Host cells transformed with a nucleotide sequence which encodes the signal transduction molecule of the present invention may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. Particularly preferred embodiments of the present invention are host cells transformed with a purified polynucleotide comprising a nucleic acid sequence to encode the polypeptide having the sequence as depicted in SEQ ID NO:3 or a contemplated variant thereof. Cells of this type or preparations made from them may be used to screen for modulators of the biological and/or pharmacological activity of the native signal transduction molecules SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

Eukaryotic recombinant host cells are especially preferred. Examples include but are not limited to yeast, mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to Drosophila and silkworm derived cell lines. Cell lines derived from mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M(TK-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616),BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The expression vector may be introduced into host cells expressing the novel GTPase effector kinase *via* any one of a number of techniques including but not limited to transformation, transfection, lipofection, protoplast fusion, and electroporation. Commercially available kits applicable for use with the present invention for hererologous expression, including well-characterized vectors, transfection reagents and conditions, and cell culture materials are well-established and readily available. CLONTECH, Palo Alto, CA;

INVITROGEN, Carlsbad, CA; PHARMINGEN, San Diego, CA; STRATAGENE, LaJolla, CA. The expression vector-containing cells are clonally propagated and individually analyzed to determine the level of novel kinase protein production. Identification of host cell clones which express the novel kinase may be performed by several means, including but not limited to immunological reactivity with antibodies described herein, and/or the presence of host cell-associated specific biological activity, and/or the ability to covalently cross-link specific substrate to the novel kinase with the bifunctional cross-linking reagent disuccinimidyl suberate or similar cross-linking reagents.

The signal transduction molecule of the present invention may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals (Porath, J., Protein Exp. Purif. 3:263 (1992)), protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor XA or enterokinase (Invitrogen, San Diego CA) between the purification domain and the coding region is useful to facilitate purification.

Systems such as the CLONTECH, TALON™ nondenaturing protein purification kit for purifying 6xHis-tagged proteins under native conditions and protocols are preferred which are commercially available. CLONTECH, Palo Alto, CA.

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a nascent form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, NIH-3T3, HEK293 etc., have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express SEQ ID NO:2/SEQ ID NO:3, for

example, may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

The human signal transduction molecule can be produced in the yeast *S.cerevisiae* following the insertion of the optimal cDNA cistron into expression vectors designed to direct the intracellular or extracellular expression of the heterologous protein. In the case of intracellular expression, vectors such as EmBLyex4 or the like are ligated to the beta subunit cistron. *See, e.g.*, Rinas, U., *et al.*, Biotechnology, 8:543 (1990); Horowitz, B., *et al.*, J. Biol. Chem., 265:4189 (1989). For extracellular expression, the kinase cistron is ligated into yeast expression vectors which may employ any of a series of well-characterized secretion signals. The levels of expressed novel kinase are determined by the assays described herein.

A variety of protocols for detecting and measuring the expression of the novel molecule as well as functional derivatives thereof, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes may be employed. Well known competitive binding techniques may also be employed. See, e.g., Hampton, R., *et al.* (1990), *Serological Methods - a Laboratory Manual*, APS Press, St Paul Minn.; Maddox, D.E., *et al.*, J. Exp. Med. 158:1211.

Screening Assays

Methods are provided to screen compounds individually, or libraries of compounds, for the identification of compounds which have the ability to modulate a biological and/or pharmacological activity of a signal transduction serine threonine protein kinase, particularly the SEQ ID NO:3 GTPase effector kinase described herein. The present invention is also directed to methods of screening for compounds which modulate the expression (transcription and/or translation) of DNA or RNA encoding the novel human kinase polypeptide SEQ ID

NO:3. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules (e.g., small molecule drug compounds).

Compounds may modulate an ultimate biological and/or pharmacological activity by increasing or attenuating the expression of DNA or RNA encoding the human signal-transduction biomolecule or a function of the native SEQ ID NO:3. Compounds that modulate the expression of DNA or RNA encoding the human signal-transduction kinase polypeptide or the function of the polypeptide may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

The human signal-transduction kinase described herein, its functional fragments or oligopeptides can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The fragment or entity employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The abolition or modulation of activity or the formation of binding complexes, between the human signal-transduction GTPase effector kinase and the agent being tested, may be measured, for example, by means provided (see, Examples II, VI, VII). Accordingly, the present invention provides a method for screening a plurality of compounds for specific binding affinity with the native polypeptide SEQ ID NO:3 or a variant thereof contemplated herein, comprising providing a plurality of compounds; combining an embodiment of the human signal-transduction kinase of the present invention with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and detecting binding of the kinase polypeptide, or fragment thereof, to each of the plurality of compounds, thereby identifying the compounds which specifically bind the human signal-transduction kinase polypeptide.

Methods of identifying compounds that modulate a biological and/or pharmacological activity of a signal-transduction molecule are generally preferred, which comprise combining a candidate compound modulator of signal transduction activity with a polypeptide having the sequence as depicted in SEQ ID NO:3 or a variant thereof contemplated herein, and measuring an effect of the candidate compound modulator on the biological and/or pharmacological activity of the polypeptide.

Methods of identifying compounds that modulate a biological and/or pharmacological activity of a signal-transduction molecule are particularly preferred which comprise combining a candidate compound modulator with a host-cell expressing a polypeptide having the sequence as depicted in SEQ ID NO:3 or a variant thereof contemplated herein and measuring an effect of the candidate compound modulator on the biological and/or pharmacological activity of the polypeptide. Preferred cellular assays for inhibitors of the subject GTPase effector kinase fall into two general categories: 1) direct measurement of a biological activity, and 2) measurement of downstream events in the signaling cascade including cell/tissue/organism physiological manifestations.

In order to measure cellular activity of the GTPase effector kinase, the source may be a whole cell lysate, prepared by one to three freeze-thaw cycles in the presence of standard protease inhibitors. Alternatively, the kinase may be partially or completely purified by standard protein purification methods. Finally, the kinase may be purified by affinity chromatography using antibodies described herein or by ligands specific for the epitope tag engineered into the recombinant kinase moreover described herein. The preparation may then be assayed for activity as described, for example, in Examples II, VI, VII.

A filter assay based on the protocol of Reuter *et al.* (1995) is also used to screen for compounds which modulate kinase activity, for example, of SEQ ID NO:3. *See*, Example VI.

In another embodiment of the invention to identify agents which modulate a biological activity of the novel biomolecule set forth herein, a nucleic acid sequence which encodes a human signal-transduction molecule as depicted in SEQ ID NO:3 may be ligated to a heterologous sequence to encode a fusion protein for use in a yeast 2-hybrid system. To screen compounds for the modulation of SEQ ID NO:3 biological activity, it is necessary to encode a chimeric kinase molecule as described herein for expression in hererologous host cells. Chimeric constructs are also used to express a 'bait', according to methods well known using a yeast two-hybrid system, using accessory native peptides that are expected to be associated with the novel human signal-transduction kinase molecule described herein, e.g., small rho-like GTPases, p21 GTPase, rac1, Cdc42, adaptor proteins which contains at least one Src-homolog 3 (SH3) domain, or the adaptor protein Nck. The two-hybrid system uses the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA-binding site that regulates the expression of an adjacent reporter gene.

Compounds which are able to modulate the biological activity of the novel biomolecule as defined herein are identified by the their ability to effect protein:protein interactions (reconstitution of the chimeric transcriptional activators) and hence the yeast 2-hybrid readout assays well-known to artisans of ordinary skill in this area of molecular biology. Fields, S., *et al.*, Trends Genet., 10:286 (1994); Allen, J.B., *et al.*, TIBS, 20:511 (1995). Fields, S., Song, O., Nature 340:245 (1989). Commercially available systems such as the CLONTECH, Matchmaker™ systems and protocols may be used with the present invention. CLONTECH, Palo Alto, CA. See also, Mendelsohn, A.R., Brent, R., Curr. Op. Biotech., 5:482 (1994); Phizicky. E.M., Fields, S., Microbiological Rev., 59(1):94 (1995); Yang, M., et al., Nucleic Acids Res., 23(7):1152 (1995); Fields, S., Sternglanz, R., TIG, 10(8):286 (1994); and US Patents 5,283,173, *System to Detect Protein-Protein Interactions*, and 5,468,614, which are incorporated herein by reference.

To further evaluate the ability of a compound to modulate the pharmacological activity, for example, of SEQ ID NO:3, human tumor cells are injected into SCID mice (severe combined immunodeficiency) to form palpable tumor masses. *See*, Example V.

Compounds which are identified generally according to methods described, contemplated, and referenced herein that modulate a biological and/or pharmacological activity of a human signal-transduction molecule of the sequence as depicted in SEQ ID NO:3 are especially preferred embodiments of the present invention.

An especially preferred embodiment of the present invention is a method for treatment of a patient in need of such treatment for a condition which is mediated by the human signal-transduction molecule described herein, e.g., SEQ ID NO:3, comprising administration of a therapeutically effective amount of a human signal-transduction modulating compound identified using sequences as depicted in SEQ ID NO:2 and/or SEQ ID NO:3 or a contemplated variant thereof as a pharmacological target in methods contemplated herein.

A method of modulating a biological and/or pharmacological activity of a signal transduction kinase in a cell, tissue, or organism is preferred which comprises administering an effective amount of a polynucleotide contemplated herein.

Antibodies

An example for the production of effective polyclonal antibodies against peptides derived from SEQ ID NO:3, for employment in methods described herein, is described in

Example III. Monospecific antibodies to the signal transduction kinase polypeptide of the present invention are purified from mammalian antisera containing antibodies reactive against the polypeptide or are prepared as monoclonal antibodies reactive with the signal transduction polypeptide using the technique of Kohler and Milstein, Nature, 256:495 (1975). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for the novel signal transduction molecule. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated with the signal transduction kinase, as described. GTPase effector kinase specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with rabbits being preferred, with an appropriate concentration of the human signal transduction kinase either with or without an immune adjuvant. Monoclonal antibodies are produced *in vivo* by injection of pristane primed Balb/c mice, approximately 0.5 ml per mouse, with about 2 x 10^6 to about 6 x 10^6 hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer and the monoclonal antibodies are purified by techniques known in the art. *In vitro* production of the anti- human kinase polypeptide mAb is carried out by growing the hydridoma in DMEM containing about 2% fetal calf serum to obtain sufficient quantities of the specific mAb. The mAb are purified by techniques known in the art.

Purification of SEQ ID NO:3 *via* Affinity Columns

It is readily apparent to those skilled in the art that methods for producing antibodies may be utilized to produce antibodies specific for the signal transduction polypeptide fragments, or the full-length nascent polypeptide, e.g., SEQ ID NO:3. Specifically, it is readily apparent to those skilled in the art that antibodies may be generated which are specific for the fully functional protein or fragments thereof.

Signal transduction polypeptide antibody affinity columns are made by adding the antibodies to Affigel-10 (Biorad), a gel support which is activated with N hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in phosphate buffered saline (pH 7.3)

with appropriate detergent and the cell culture supernatants or cell extracts containing human signal-transduction kinase polypeptide made using appropriate membrane solubilizing detergents are slowly passed through the column. The column is then washed with phosphate buffered saline/detergent until the optical density falls to background, then the protein is eluted with 0.23M glycine-HCl (pH 2.6)/detergent. The purified signal-transduction kinase polypeptide is then dialyzed against phosphate buffered saline/detergent.

Recombinant GTPase effector kinase molecules can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full length nascent signal transduction polypeptide, or polypeptide fragments of the kinase molecule.

GTPase effector signal transduction kinase polypeptides described herein may be used to affinity purify biological effectors from native biological materials, e.g. disease tissue. Affinity chromatography techniques are well known to those skilled in the art. A signal-transduction peptide described herein or an effective fragment thereof, is fixed to a solid matrix, e.g. CNBr activated Sepharose according to the protocol of the supplier (Pharmacia, Piscataway, NJ), and a homogenized/buffered cellular solution containing a potential molecule of interest is passed through the column. After washing, the column retains only the biological effector which is subsequently eluted, e.g., using 0.5M acetic acid or a NaCl gradient.

Immunoprecipitation is described in Example IV.

<u>Diagnostic Assays</u>

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay (RIA) techniques. Similar diagnostic assays are used to detect the presence of the novel signal transduction kinase polypeptide in body fluids or tissue and cell extracts.

Diagnostic assays using the human signal-transduction polypeptide specific antibodies are useful for the diagnosis of conditions, disorders or diseases characterized by abnormal expression of the signal-transduction biomolecule or expression of genes associated with abnormal cell growth. Diagnostic assays for the signal-transduction kinase of this invention

include methods utilizing the antibody and a label to detect the human kinase polypeptide in human body fluids, cells, tissues or sections or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule, a myriad of which are well-known to those skilled in the art.

A variety of protocols for measuring the kinase polypeptide, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the human signal-transduction kinase polypeptide is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, D.E. *et al.*, J. Exp. Med. 158:1211 (1983); Sites, D.P., *et al.*, *Basic and Clinical Immunology*, Ch.22, 4th Ed., Lange Medical Publications, Los Altos, CA (1982); U.S. Patents No. 3,654,090, No. 3,850,752; and No. 4,016,043.

In order to provide a basis for the diagnosis of disease, normal or standard values for the signal-transduction molecule, normal expression levels must be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with antibody to the human kinase polypeptide under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by comparing it with a dilution series of positive controls where a known amount of antibody is combined with known concentrations of purified human signal-transduction kinase polypeptide. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by a disorder or disease related to the human kinase polypeptide expression. Deviation between standard and subject values establishes the presence of the disease state.

Kits containing a signal-transduction kinase nucleic acid, antibodies to a corresponding polypeptide, or protein, e.g., SEQ ID NO:3 may be prepared. Such kits are used to detect heterologous nucleic acid which hybridizes to the signal transduction nucleic acid, or to detect the presence of protein or peptide fragments in a sample. Such

characterization is useful for a variety of purposes including, but not limited to, diagnosis of pathophysiological conditions, forensic analyses, and epidemiological studies.

The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of the novel kinase DNA, RNA or protein. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of the signal-transduction GTPase effector kinase. Such a kit comprises a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant kinase or anti-kinase antibodies suitable for detecting the novel molecule as depicted in SEQ ID NO:3. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

Polynucleotide sequences which encode the transduction molecule may be used for the diagnosis of conditions or diseases with which the expression of the novel human stress-activated kinase is associated. For example, polynucleotide sequences encoding the signal-transduction molecule may be used in hybridization or PCR assays of fluids or tissues from biopsies to detect expression of the kinase. The form of such qualitative or quantitative methods may include Southern or northern analysis, dot blot or other membrane-based technologies; PCR technologies; dip stick, pin, chip and ELISA technologies. All of these techniques are well known in the art and are the basis of many commercially available diagnostic kits. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regime in animal studies, in clinical trials, or in monitoring the treatment of an individual patient. Once disease is established, a therapeutic agent is administered and a treatment profile is generated. Such assays may be repeated on a regular basis to evaluate whether the values in the profile progress toward or return to the normal or standard pattern. Successive treatment profiles may be used to show the efficacy of treatment over a period of several days or several months.

Polynucleotide sequences which encode the novel kinase may also be employed in analyses to map chromosomal locations, e.g., screening for functional association with disease markers. Moreover the sequences described herein are contemplated for use to identify human sequence polymorphisms and possible association with disease as well as analyses to select optimal sequence from among possible polymorphic sequences for the design of

compounds to modulate the biological and/or pharmacological activity. Furthermore the sequences are contemplated as screening tools for use in the identification of appropriate human subjects and patients for therapeutic clinical trials.

PCR Diagnostics

The nucleic acid sequence, oligonucleotides, fragments, portions or antisense molecules thereof, may be used in diagnostic assays of body fluids or biopsied tissues to detect the expression level of the novel human signal-transduction kinase molecule. For example, sequences designed from the cDNA sequence SEQ ID NO:1 or sequences comprised in SEQ ID NO:2 can be used to detect the presence of the mRNA transcripts in a patient or to monitor the modulation of transcripts during treatment. *See*, Example VIII.

One method for amplification of target nucleic acids, or for later analysis by hybridization assays, is known as the polymerase chain reaction ("PCR") or PCR technique. The PCR technique can be applied to detect sequences of the invention in suspected samples using oligonucleotide primers spaced apart from each other and based on the genetic sequence, e.g., SEQ ID NO:1, set forth herein. The primers are complementary to opposite strands of a double stranded DNA molecule and are typically separated by from about 50 to 450 nucleotides or more (usually not more than 2000 nucleotides). This method entails preparing the specific oligonucleotide primers followed by repeated cycles of target DNA denaturation, primer binding, and extension with a DNA polymerase to obtain DNA fragments of the expected length based on the primer spacing. One example embodiment of the present invention is a diagnostic composition for the identification of a polynucleotide sequence comprising the sequence as depicted in SEQ ID NO:2 comprising PCR primers derived from SEQ ID NO:1. The degree of amplification of a target sequence is controlled by the number of cycles that are performed and is theoretically calculated by the simple formula 2n where n is the number of cycles. *See, e.g.*, Perkin Elmer, *PCR Bibliography*, Roche Molecular Systems, Branchburg, New Jersey; CLONTECH products, Palo Alto, CA; U.S. Patent No. 5,629,158, *Solid Phase Diagnosis of Medical Conditions*, issued May 13, 1997.

Compositions

Pharmaceutically useful therapeutic compositions which comprise a nucleic acid coding region, a dominant negative mutant coding region, an antisense sequence, a polypeptide as depicted in SEQ ID NO:3 or a variation thereof contemplated herein, or a

compound that modulates the biological and/or pharmacological activity of the novel signal transduction biomolecule set forth herein may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in *Remington's Pharmaceutical Sciences* (Maack Publishing Co, Easton, PA). To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein, DNA, RNA, or modulator.

Therapeutic or diagnostic compositions of the invention are administered to an individual or used in amounts sufficient to treat or diagnose signal-transduction polypeptide or GTPase effector kinase related disorders. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

The term functional derivative includes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as *Remington's Pharmaceutical Sciences*.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. The therapeutically effective dose can be estimated initially either in cell culture assays, eg, of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutically effective dose refers to that amount of compound, protein, peptide, nucleic acid, antibodies, antagonists, or inhibitors which ameliorate the symptoms or condition. The exact dosage is chosen by the individual physician in view of the patient to be treated.

Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages defined by routine testing in order to obtain optimal modulation of a

signal-transduction biomolecule, or its activity while minimizing any potential toxicity. In addition, co-administration or sequential administration of other agents may be desirable.

The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular. Administration of pharmaceutical compositions is accomplished orally or parenterally. Methods of parenteral delivery include topical, intra-arterial (directly to the tissue), intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration. The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient for use in the modulation of a signal-transduction molecule can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be employed as a signal-transduction modulating agent.

The daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult human/per day. For oral administration, the compositions are preferably provided in the form of scored or unscored tablets containing 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, and 50.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. An effective amount of the drug is ordinarily supplied at a dosage level of from about 0.0001 mg/kg to about 100 mg/kg of body weight per day. The range is more particularly from about 0.001 mg/kg to 10 mg/kg of body weight per day. Even more particularly, the range varies from about 0.05 to about 1 mg/kg. Of course the dosage level will vary depending upon the potency of the particular compound. Certain compounds will be more potent than others. In addition, the dosage level will vary depending upon the bioavailability of the compound. The more bioavailable and potent the compound,

the less compound will need to be administered through any delivery route, including but not limited to oral delivery. The dosages of the human signal-transduction kinase modulators are adjusted when combined to achieve desired effects. On the other hand, dosages of these various agents may be independently optimized and combined to achieve a synergistic result wherein the pathology is reduced more than it would be if either agent were used alone. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells and conditions.

Example I - Sequence Construction

The human signal transduction serine threonine protein kinase was identified after the assembly of EST sequences from the Merck Washington University EST sequence database and a proprietary database. Two Merck Wash. U. EST clones, along with a number of other EST clones, were identified by BLAST analysis using the conserved serine/threonine kinase domain VIb motif found in a number of Mitogen activated kinase kinase kinase kinases (e.g. SOK1, MST2). Complete in-house sequence analysis of the 2 Merck Wash U ESTs (yf64a05 and ym92f08) provided a consensus sequence containing conserved kinase catalytic domains 1- XI, a short C-terminal non-catalytic region, and 3' UTR ending in a poly adenylated tail. The sequence was recognized to display similarity to the p21 Activated Kinase (PAK) family of serine/threonine kinases. Additional sequence was obtained from an ORIGENE (Rockville, MD) Placenta cDNA Array library. The library was screened via PCR using the primers generated from the assembled ESTs. A 3600bp cDNA clone (SEQ ID NO:1) was identified, sequenced in its entirety, and determined to contain an open reading frame (SEQ ID NO:2) encoding 681 amino acids (SEQ ID NO:3).

Example II - Assay for Kinase Activity

Recombinant, purified GST/SEQ ID NO:3 kinase (or other recombinant protein derived from SEQ ID NO:3) is added to 20μg myelin basic protein (MBP) in 10μL of a 3X kinase reaction buffer (KRB) containing (in mM): 60 HEPES (pH 7.5), 30 magnesium acetate, 0.15 ATP, 3 DTT, 0.03 sodium orthovanadate. The reaction is started by the addition of 5μCi [γ-^{32}P] ATP (10μL). Samples are incubated for 5 minutes at 30°C. The reaction stopped by addition of 4X Laemmli sample buffer. Proteins are separated on 12% Tris/glycine SDS gels, stained with Coomassie blue, dried and exposed to autoradiograph film.

Example III - Production of Anti-Kinase Polyclonal Antibodies

Antigenic peptide fragments were identified within the N-terminal, c-terminal and central regions of the signal transduction molecule utilizing a well established algorithm method developed by Jameson and Wolf. *The Antigenic Index: A Novel Algorithm for Predicting Antigenic Determinants*, CABIOS, 4:181 (1988). The algorithm carries out six major subroutines with the following hierarchy:

1) determination of hydrophilicity, Hopp-Woods (1981)
2) calculation of surface probability, Emini (1985)
3) prediction of backbone or chain flexibility, Karplus-Schultz (1985)
4) prediction of secondary structure, Chou-Fasman (1978)
5) prediction of secondary structure, Garnier-Robson (1978)
6) flexibility parameters and hydropathy/solvent accessibility factors are combined to determine the antigenic index

The antigenic index was plotted for the entire molecule. Two peptide sequences corresponding to SEQ ID NO:3 amino acid residue positions 51-61 as well as 287-296 were each selected for synthesis and antibody production based on antigenicity and uniqueness of sequence compared to other identified kinase family members.

Anti-peptide antisera (rabbit polyclonal) was raised against the two peptides. The peptides and antisera were produced by Cambridge Research Biochemicals (ZENECA Specialty Chemicals, 1800 Concord Pike, Wilmington, DE 19850-5457).

Chou, P.Y. and Fasman, G.D., (1978) Prediction of the secondary structure of proteins from their amino acid sequence, *Adv. Enzymol*, 47:45-148; Emini, E. A., Hughes, J., Perlow, D. and Boger, J., (1985) Induction of Hepatitis A Virus-Neutralizing Antibody by a Virus-Specific Synthetic Peptide, *J. Virology*, 55:836-839; Garnier, J., Osguthorpe, D.J., and Robson, B., (1978) Analysis of the accuracy and implications of simple method for predicting the secondary structure of globular proteins, *J.Mol. Biol.*, 120:97-120; Harlow, E. and Lane, D., (1988) *Antibodies: A Laboratory Manual.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Hopp, T. P. and Woods, K. R., (1981) Prediction of Protein Antigenic Determinants from Amino Acid Sequences, *Proc. Natl. Acad. Sci.*, 78:3824-3828; Jameson, B.A., and Wolf, H., (1988) The antigenic index: a novel algorithm for predicting

antigenic determinants, *CABIOS* 4:181-186; Karplus, P.A. and Schultz, G.E., (1985) Prediction of chain flexibility in proteins, *Naturwissenschaften*, 72:212-213.

Example IV - Immunoprecipitation

Immunoprecipitation of the signal transduction molecule described herein is performed substantially according to the method described by Suchard, S.J., *et al.* J. Immunol., 158:4961 (1997). Cell lysates are combined with 1μg of either anti-enterokinase protease cleavage site/Xpress™ antibody (Invitrogen Corp.) for the recombinant signal transduction serine threonine protein kinase described herein or peptide-specific polyclonal antibody against the native kinase described herein. Rabbit IgG is used as a control. Samples are incubated at 4°C ≥ 2 hours with rotation. Immunocomplexes are incubated with protein A Sepharose (Pharmacia) for 2 hours at 4°C with rotation. The beads are washed in buffer containing 50 mM Tris (pH 8.0), 100 mM NaCl, 1 mM Na_3VO_4, 1% Triton X-100, and Complete™ Protease Inhibitor Cocktail. Adsorbed proteins are solubilized in sample buffer and separated on 12% SDS-PAGE minigels.

Example V - Efficacy (Pharmacological Activity) Screen

To further evaluate the ability of a compound to inhibit human tumor growth, for example, human tumor cells are injected into SCID mice (severe combined immunodeficiency) to form palpable tumor masses. The effects of various doses (e.g., 1-50mg) of a compound in inhibiting tumor growth can be determined as follows: approximately 1 x 10^7cells of the CCL 221 cell line (ATCC, Rockville, Md.), a human ras-dependent colon adenocarcinoma cell line, is suspended in 100 μl DMEM and injected subcutaneously into SCID mice, such that two tumors per mouse are formed. SCID mice receive CCL 221 cells and the tumors are grown for 7 days without treatment; on the 7th day (Day 0) tumor maximal diameters and animal weights are recorded and the mean tumor size for the mice is determined. On Day 1 (eight days following tumor cell injection), treatment of the mice with the candidate compound or vehicle alone is begun. One group of the mice (controls) are injected intraperitoneally with 0.2 ml of vehicle and a second group of mice receives compound by intraperitoneal injection. Various doses (0.25-75mg) of the compound can be tested in separate groups of mice. On Day 7 and Day 14, animal weight and maximal tumor diameter is measured. Average maximal tumor size for each group on Day 0, Day 7, and Day 14 are compared Day 14, one high dose animal is followed for an additional to

determine whether the agent produces a dose-dependent inhibition of tumor growth. Toxicity effects can be examined by tracking mice weight and by harvesting lungs, livers, and spleens of the animals for histological staining.

Example VI - High Throughput Screening For Compounds Which Modulate Activity

High throughput screening for modulator compounds is performed using MBP coated 96-well FlashPlates® (NEN™ Life Science Products). Kinase reaction buffer (3X kinase reaction buffer (KRB) contains: 60 mM HEPES (pH 7.5), 30 mM magnesium acetate, 0.15 mM ATP, 3 mM DTT, 0.03 mM sodium orthovanadate) 0.25μCi [γ ^{33}P]-ATP at a concentration no greater than 1 μg/ml, (determined by titration of individual enzyme preparations for a concentration that allows kinetic determinations over a 1 hour time course of the human kinase) are added to each well and incubated 1 hour at 30°C in the presence or absence of 10μM test compound. Total reaction volume is 100μL. Following incubation, the reaction mixture is aspirated and the wells rinsed 2 times with 300μL PBS. Incorporation of raiolabeled phosphate is determined by scintillation counting, Packard Instrument Co.TopCount, 12-detector, 96-well microplate scintillation counter and luminescence counter, model B991200. Compounds which inhibit kinase activity ≥50 percent at 10μM are indicated by a >50% reduction in scintillation counts. Specificity and selectivity are determined by titration of inhibitory compounds to determine the IC_{50} (or other standard quantitation well known in the art for comparison) and by the substitution of other kinases in the assay. For example, determination of relative inhibitory activity of the kinase in comparison to recombinant PAK1, PAK2, SOK-1 and/or mst-3, expressed and isolated in a similar manner, assayed under similar conditions, provides selectivity data.

Alternatively, a filter assay based on the protocol of Reuter *et al.* (1995) may be used. This protocol is the same but the reaction is stopped by the addition of EDTA (pH 7.0) to a final concentration of 80mM. Samples are then centrifuged and 50μL of the supernatant spotted on p81 cation-exchange filter paper (Whatman, No. 3698 915). The filters are then washed 3 times in 200mL of 180mM H_3PO_4 (5-10min each) and once in 200mL of 96% ethanol. After air drying the filters, radioactivity is determined by Cerenkov counting in a scintillation counter. Reuter, C.W.M., Catling, A.D. and Weber, M.J., *Immune Complex Kinase Assays for Mitogen-Activated Protein Kinase and MEK*, Methods In Enzymology, 255:245 (1995).

Example VII - High Throughput Screening Protocol

Test compounds Test compounds are prepared in advance from 2.5 mg/ml stock solutions in DMSO by diluting 1:10 in distilled water and then 1:10 again. Ten (10)µl of the 1:100 dilution solutions (25 µg/ml in 1% DMSO) are prepared in 96 well Microlite 1 plates (Dynatech) and plates are stored at -20°C until the evening prior to the start of the assay.

Control plates A plate containing control solutions is included in each run of the screen for QA purposes. Such plates are prepared at the beginning of the HTS campaign and stored at -20°C until required. Zero inhibition (MAX. signal) wells (columns 3, 6, 8 and 10) contain 10 µl of 1% (v/v) DMSO solution in MilliQ water. 100% inhibition (MIN signal) wells (columns 1, 4, 9 and 11) contain 10 µl of 220 nM ZM333141/1 in 1% DMSO solution in MilliQ water. 50% inhibition (REF. signal) wells (columns 2, 5, 7 and 12) contain a reference compound at a concentration known to provide approximately 50% inhibition in 1% (v/v) DMSO solution in MilliQ water.

Assay components (1) recombinant kinase (expressed in *E.coli* or eukaryotic cells as described herein) or a lysate of a prokaryotic or eukaryotic cell expressing recombinant enzyme, or the natural enzyme partially purified from a human cell line.

(2) [γ-^{33}-P]-adenosine triphosphate

(3) myelin basic protein linked to the surface of PVT SPA beads (purchased from Amersham International) by an antibody-protein A or other appropriate method.

To Microlite I plates containing 10µl of test compound, which have been left on the bench overnight to reach room temperature, 25ml of GST-Rb/ATP/ATP^{33} is added, immediately followed by 20µl of Enzyme, using two Multidrops. The plates are stacked in 13 plate stacks (with an empty plate on top of each stack to minimise evaporation from the top plate) and left at room temperature for 105 minutes. 150µl of "Stop Solution" containing beads antibody and EDTA is added using a Multidrop. The plates are sealed with Topseal-S plate sealers and left on the bench overnight, surrounded by Scotlab perspex screens. The plates are then centrifuged (Heraeus Megafuge 3.0R) at 2500rpm, 1124xg., for 5 minutes (2 plates per trunnion) and counted on a Topcount (I4.34); (isotope:P^{33}; counting time: 20 seconds/well).

The data may be analysed using well-known software systems. A threshold for inhibition is set, e.g., 60% inhibition of scintillation signal. Compounds reaching the inhibition threshold are scored as active.

Example VIII - PCR of SEQ ID NO:2

The human signal transduction serine threonine protein kinase coding region (SEQ ID NO:2) can be obtained from existing cDNA (e.g., ORIGENE (Rockville, MD) Placenta cDNA library or other suitable cDNA source) by PCR ampflication using the following primers: Sense 5'- GCCCCGAATTCGGCACCATGTTCCGCAAG-3' (SEQ ID NO:6) and Antisense 5'- CTCGTGGCGGCCGCTCAGCAGGTGGAGGTCTG-3' (SEQ ID NO:7). The resulting PCR amplicon can be subcloned into a vector using the encoded (integral to the primers outside of the SEQ ID NO:2 cDNA sequence) EcoRI (5') and NotI (3') restriction sites.

Example IX - Expression And Purification Of Gst/Human Signal Transduction Serine Threonine Protein Kinase

A single, isolated, BL21 transformed clone is grown overnight in 10mL Lennox L broth (LB broth containing 50mg/ml carbenicillin) and then seeded into 1 liter LB broth/carbenicillin and grown at 37°C with shaking (225rpm) to an A_{600} of 0.5-0.8. Expression of GST/protein kinase fusion protein is induced by adding isopropylthio-β-galactoside to 100mM and continuing the incubation for 2 additional hours. Following incubation, the cells are centrifuged 1500 x *g* for 10 min at 4°C, resuspended in 50mL phosphate buffered saline (PBS) containing Complete™ Protease Inhibitor Cocktail (Boehringer Mannheim GmbH), then lysed by sonication on ice. Triton X-100 is added to the sonicate to a final concentration of 1% to aid in the solubilization of the fusion protein. Cellular debris is removed by centrifugation (12,000 x *g*, 4°C) and the supernatant is used as the source for obtaining purified GST/kinase.

Purification of GST/Protein Kinase Fusion Protein

GST/protein kinase is purified by Glutathione Sepharose 4B beads (Pharmacia) affinity column chromatography using a 1ml gravity fed open column. The suspension is allowed to pass through the column then the column was washed three times with PBS containing protease inhibitors. Finally, the GST-kinase fusion protein is eluted from the column by the addition of 1mL elution buffer (10mM reduced glutathione in 50mM Tris-HCl, pH 8.0 with protease inhibitors). The GST/kinase is stored in aliquots at -20°C until needed.

Eample X - Example Dominant Negative Mutant SEQ ID NO:8

Mutation of SEQ ID NO:3 lysine at position 436 to arginine results in SEQ ID NO:8 which is catalytically inactive. Primers (sense 5'-CAG GTG GCC GTC CGG ATG ATG GAC CTC-3' (SEQ ID NO:9); and antisense 5'-GAG GTC CAT CAT CCG GAC GGC CAC CTG-3' (SEQ ID NO:10)) may be used to synthesize the dominant negative mutant (SEQ ID NO:8) coding region. The mutation may be obtained, for example, using STRATAGENE, LaJolla, CA, Quick Change Site-Directed Mutagenesis kit using the primers. The product cDNA may be inserted into expression vectors with or without fusion protein "tags" for expression of recombinant protein which can be purified. Example commercially available prokaryotic expression vectors such as pGEX (GST fusion), pET (+/- His_6 or T7 tag) as well as commercially available eucaryotic expression vectors including the likes of pcDNA3.1His, pIRES-EGFP, pcDNA3, pEBVHis, each of which are well known in the art, for example, may be used to produce this as well as other dominant negative mutants contemplated herein.

Example XI - Example Dominant Negative Mutant SEQ ID NO:11

Mutation of lysine 436 to arginine as well as of histidine 20 to leucine and histidine 23 to leucine results in SEQ ID NO:11 which is expected to lack the biological ability to bind to family members of small rho-like GTPases, including, but not limited to, the likes of p21, rac1, Cdc42. The mutation may be obtained, for example, using STRATAGENE, LaJolla, CA, Quick Change Site-Directed Mutagenesis kit using the primers: sense 5'- CAG AAC TTC CAG CTG CGT GTC CTC ACC TCC TTC GAC -3' (SEQ ID NO:12); and, antisense 5'- GTC GAA GGA GGT GAG GAC ACG CAG CTG GAA GTT CTG -3' (SEQ ID NO:13) to produce the H20L and H23L mutations on a cDNA containing the previously described (Example X) K436R mutation.

* * *

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of

the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Claims

1. A purified polynucleotide comprising a nucleic acid sequence which encodes a polypeptide comprising the sequence as depicted in SEQ ID NO:3 or a variant of SEQ ID NO:3 having at least about 80% homology to a member selected from the group consisting essentially of: (SEQ ID NO:3, SEQ ID NO:3 positions 1-413, SEQ ID NO:3 positions 414-653, SEQ ID NO:8, SEQ ID NO:8 positions 414-653, SEQ ID NO:11, SEQ ID NO:11 positions 1-413).

2. The polynucleotide of Claim 1 wherein the polynucleotide sequence comprises the sequence as depicted in SEQ ID NO:2.

3. An expression vector comprising the polynucleotide of Claim 1.

4. An antisense molecule comprising an oligomer in the range from about 12 to about 25 nucleotides in length which: (a) is complementary to a region within positions 97-178 of SEQ ID NO:1; or, (b) comprises a sequence which is complementary to a sequence selected from the group consisting of: (SEQ ID NO:1 positions 97-109; 100-112; 116-128; 123-135; 124-136; 125-137; 126-138; 127-139; 128-140; 129-141; 130-142; 131-143; 132-144; 133-145; 134-146; 135-147; 136-148; 137-149; 138-150; 139-151; 140-152; 141-153; 142-154; 143-155; 144-156; 145-157; 146-158; 147-159; 148-160; 149-161; 150-162; 151-163; 152-164; 153-165; 154-166; 155-167; 156-168; 157-169; 158-170; and 166-178).

5. A host cell transformed with the expression vector of Claim 3.

6. A purified polypeptide comprising the amino acid sequence as depicted in SEQ ID NO:3 or a variant of SEQ ID NO:3 having at least about 80% homology to a member selected from the group consisting essentially of: (SEQ ID NO:3, SEQ ID NO:3 positions 1-413, SEQ ID NO:3 positions 414-653, SEQ ID NO:8, SEQ ID NO:8 positions 414-653, SEQ ID NO:11, SEQ ID NO:11 positions 1-413).

7. An antibody specific for the polypeptide according to Claim 6.

8. A method for producing cells which express a polypeptide according to Claim 6, said method comprising:

a) culturing a host cell according to Claim 5 under conditions suitable for the expression of said polypeptide.

9. A method for producing a polypeptide according to Claim 6, said method comprising the steps of:

a) culturing a host cell according to Claim 5 under conditions suitable for the expression of said polypeptide, and

b) recovering said polypeptide from the host cell culture.

10. A method of identifying compounds that modulate a biological and/or pharmacological activity of a signal-transduction kinase, comprising:

(a) combining a candidate compound modulator with a polypeptide according to Claim 6, and

(b) measuring an effect of the candidate compound modulator on the biological and/or pharmacological activity of the polypeptide.

11. A method of identifying compounds that modulate a biological and/or pharmacological activity of a signal-transduction kinase according to Claim 10, comprising:

(a) combining a candidate compound modulator with a host-cell expressing a polypeptide according to Claim 6, and

(b) measuring an effect of the candidate compound modulator on the biological and/or pharmacological activity of the polypeptide.

12. A compound that modulates the activity of a signal-transduction kinase identified by the method of Claim 10.

13. A pharmaceutical composition comprising a compound that modulates the activity of a signal-transduction kinase identified by the method of of Claim 10.

14. A method of treatment of a patient in need of such treatment for a condition which is mediated by a signal-transduction kinase, comprising administering an effective amount of a compound according to Claim12.

15. A method of modulating a biological and/or pharmacological activity of a signal transduction kinase in a cell comprising administering an effective amount of a polynucleotide according to Claim 1 to said cell.

16. A method for modulating the expression of a signal-transduction kinase in a cell comprising administering an effective amount of the antisense molecule of Claim 4 to said cell.

17. A diagnostic composition, for the identification of a polypeptide comprising the amino acid sequence as depicted in SEQ ID NO:3, comprising the antibody of Claim 7.

18. A diagnostic composition for the identification of a polynucleotide sequence comprising the sequence as depicted in SEQ ID NO:2 comprising PCR primers derived from SEQ ID NO:1.

Figure 1

```
TEN-2 MF------------------------------------------------ 2
Pak1  MSNNGLDIQDKPPAPPMRNTSTMIGVGSKDAGTLNHGSKPLPPNPEEKKK 50
Pak2  MSDNG-ELEDKPPAPPVRMSSTIFSTGGKDPLSANHSLKPLPSVPEEKKP 49

TEN-2 ---------------RKKKKKRPEISAPQNFQHRVHTSFDPKEGKFVGLP 37
Pak1  KDRFYRSILPGDKTNKKKEKERPEISLPSDFEHTIHVGFDAVTGEFTGMP 100
Pak2  RHKIISIFSGTEKGSKKKEKERPEISPPSDFEHTIHVGFDAVTGEFTGMP 99

TEN-2 PQWQNILDTLRRPKPVVDPSRITRVQLQPMKTVVRGSAMPVDGYISGLLN 87
Pak1  EQWARLLQT----------SNITKSEQKKNPQAV---------------- 124
Pak2  EQWARLLQT----------SNITKLEQKKNPQAV---------------- 123

TEN-2 DIQKLSVISSNTLRGRSPTSRRRAQSLGLLGDEHWATDPDMYLQSPQSER 137
Pak1  -LDVLEFYNSKK-----------------------TSNSQKYMSFTDKSA 150
Pak2  -LDVLKFYDSNT-----------------------VK--QKYLSFTPPEK 147

TEN-2 TD-PHGLYLSCNGGTPAGHKQMPWPEPQSPRVLPNGLAAKAQSLGPAEFQ 186
Pak1  ED-YNS---------------------------SNALNVKAVSETPA--- 169
Pak2  DGLPSG---------------------------TPALNAKG-----T--- 162

TEN-2 GASQRCLQLGACLQSSPPGASPPTGTNRHGMKAAKHGSEEARPQSCLVGS 236
Pak1  --------------------VPPVSED------EDDDDDDATPPPVI--- 190
Pak2  --------------------EAPAVVT------EEEDDDEETAPPVI--- 183

TEN-2 ATGRPGGEGSPSPKTRESSLKRRLFRSMFLSTAATAPPSSSKPGPPPQSK 286
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Pak2  ------------------------------------------D------- 212

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TEN-2 GALTDIVSQV-RLNEEQIATVCEAVLQALAYLHAQGVIHRDIKSDSILLT 535
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TEN-2 LDGRVKLSDFGFCAQISKDVPKRKSLVGTPYWMAPEVISRSLYATEVDIW 585
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TEN-2 SLGIMVIEMVDGEPPYFSDSPVQAMKRLRDSPPPKLKNSHKVSPVLRDFL 635
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Pak2  SLGIMAIEMVEGEPPYLNENPLRALYLIATNGTPELQNPEKLSPIFRDFL 478

TEN-2 ERMLVRDPQERATAQELLDHPFL-LQTGLPECLVPLIQLYRKQTSTC.  682
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Pak2  NRCLEMDVEKRGSAKELLQHPFLKLAKPLSS-LTPLIMAAKEAMKSNR   525
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Decoration 'Decoration #1': Box residues that match TEN-2 exactly.

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gaccccttga tatttgcaca gggatatttc taagaaacgc agaggccagc gttcctggcc 2460
tctgcagcca acacagtaga aaaggctgct gtggtttttt aaaggcagtt gtccactagt 2520
gtcctaggcc actgcagagg gcagactgct ggtctccaca gatacctgct gttctcagct 2580

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ccagcttcaa acctcgagtc tcgagagggc cacggggtgg tttttatgac cggaatcccg     2640
cttcctccct cacgtctgat gtcctgaagg tgcagtccca cctgtacagc ccctccccgc     2700
ccagaactgt gaatggcctg ctccaggcca tggctggggg cagggagtga ggggacaatt     2760
tctgagtgaa agagaaagaa tggggtcggt ggtgaaggtg ctctcacttt acagaatgga     2820
gagaacatcg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtaag     2880
gggaggaaag ccaccttgac agcccaggtc cctccaggtc acccacagcc agtttcagga     2940
aggctgcccc tctctcccac taagttctgg cctgaaggga cctgctttct tggcctggct     3000
tccacctctc cactcctgtg tctacctggc cagtggagtg gtccatgcta agtctaacac     3060
tcctgggagc tcaggaggct tctgagcttc tcctgtactg tgcatcgtga gggccagaga     3120
caggaatgta aggattggca actgtgttac ctttcaagtt tatctcaata accaggtcat     3180
cagggaccca ttgttctctt cagaacccta tctgggagag aaggcgaacc acctccgggt     3240
ttccatcatg tcaaggtcac aggcatccat gtgtgcaaac catctgcccc agctgcctcc     3300
acagactgct gtctccttgt cctcctcggc cctgccccac ttcagggctg ctgtgagatg     3360
gaattccagg aaagaacttc aggtgtctgg acccttteta tctagataat atttttagat     3420
tcttctgctc cctagtgacc tacctggggg caaagaaatt gcaaggactt tttttaagg     3480
gtcagagttt tcaaaacaaa agcatcttcc ctagaaattt ttgtgaattg tttgcacttg     3540
tgcctgtttt aaattaaatt gagtgttcaa agccaaaaaa aaaaaaaaaa aaaaaaaaaa     3600
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<210> 2
<211> 2046
<212> DNA
<213> Homo Sapiens

<400> 2

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atgttccgca agaaaaagaa gaaacgccct gagatctcag cgccacagaa cttccagcac       60
cgtgtccaca cctccttcga ccccaaagaa ggcaagtttg tgggcctccc cccacaatgg      120
cagaacatcc tggacacact gcggcgcccc aagcccgtgg tggacccttc gcgaatcaca      180
cgggtgcagc tccagcccat gaagacagtg gtgcggggca gcgcgatgcc tgtggatggc      240
tacatctcgg ggctgctcaa cgacatccag aagttgtcag tcatcagctc caacaccctg      300
cgtggccgca gccccaccag ccggcggcgg gcacagtccc tggggctgct gggggatgag      360
cactgggcca ccgacccaga catgtacctc cagagccccc agtctgagcg cactgacccc      420
cacggcctct acctcagctg caacgggggc acaccagcag gccacaagca gatgccgtgg      480
cccgagccac agagcccacg ggtcctgccc aatggctgg ctgcaaaggc acagtccctg      540
ggccccgccg agtttcaggg tgcctcgcag cgctgtctgc agctgggtgc ctgcctgcag      600
agctccccac caggagcctc gcccccccacg ggcaccaata ggcatggaat gaaggctgcc      660
aagcatggct ctgaggaggc ccggccacag tcctgcctgg tgggctcagc cacaggcagg      720
ccaggtgggg aaggcagccc tagccctaag acccgggaga gcagcctgaa gcgcaggcta      780
ttccgaagca tgttcctgtc cactgctgcc acagcccctc caagcagcag caagccaggc      840
cctccaccac agagcaagcc caactcctct ttccgaccgc cgcagaaaga caacccccca      900
agcctggtgg ccaaggccca gtccttgccc tcggaccagc cggtggggac cttcagccct      960
ctgaccactt cggataccag cagcccccag aagtccctcc gcacagcccc ggccacaggc     1020
cagcttccag gccggtcttc cccagcggga tccccccgca cctggcacgc ccagatcagc     1080
accagcaacc tgtacctgcc ccaggacccc acggttgcca agggtgccct ggctggtgag     1140
gacacaggtg ttgtgacaca tgagcagttc aaggctgcgc tcaggatggt ggtggaccag     1200
ggtgaccccc ggctgctgct ggacagctac gtgaagattg gcgagggctc caccggcatc     1260
gtctgcttgg cccgggagaa gcactcgggc cgccaggtgg ccgtcaagat gatggacctc     1320
aggaagcagc agcgcaggga gctgctcttc aacgaggtgg tgatcatgcg ggactaccag     1380
cacttcaacg tggtggagat gtacaagagc tacctggtgg gcgaggagct gtgggtgctc     1440
atggagttcc tgcagggagg agccctcaca gacatcgtct cccaagtcag gctgaatgag     1500
gagcagattg ccactgtgtg tgaggctgtg ctgcaggccc tggcctacct gcatgctcag     1560
ggtgtcatcc accgggacat caagagtgac tccatcctgc tgaccctcga tggcagggtg     1620
aagctctcgg acttcggatt ctgtgctcag atcagcaaag acgtccctaa gaggaagtcc     1680
ctggtgggaa cccccctactg gatggctcct gaagtgatct ccaggtcttt gtatgccact     1740
gaggtggata tctggtctct gggcatcatg gtgattgaga tggtagatgg ggagccaccg     1800
tacttcagtg actccccagt gcaagccatg aagaggctcc gggacagccc ccccacccaag     1860
ctgaaaaact ctcacaaggt ctccccagtg ctgcgagact tcctggagcg gatgctggtg     1920
cgggaccccc aagagagagc cacagcccag gagctcctag accacccctt cctgctgcag     1980
acagggctac ctgagtgcct ggtgcccctg atccagctct accgaaagca gacctccacc     2040
tgctga                                                                2046
```

<210> 3
<211> 681
<212> PRT

<213> Homo Sapiens

<400> 3

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Met Phe Arg Lys Lys Lys Lys Lys Arg Pro Glu Ile Ser Ala Pro Gln
1               5                   10                  15
Asn Phe Gln His Arg Val His Thr Ser Phe Asp Pro Lys Glu Gly Lys
            20                  25                  30
Phe Val Gly Leu Pro Pro Gln Trp Gln Asn Ile Leu Asp Thr Leu Arg
        35                  40                  45
Arg Pro Lys Pro Val Val Asp Pro Ser Arg Ile Thr Arg Val Gln Leu
    50                  55                  60
Gln Pro Met Lys Thr Val Val Arg Gly Ser Ala Met Pro Val Asp Gly
65                  70                  75                  80
Tyr Ile Ser Gly Leu Leu Asn Asp Ile Gln Lys Leu Ser Val Ile Ser
                85                  90                  95
Ser Asn Thr Leu Arg Gly Arg Ser Pro Thr Ser Arg Arg Arg Ala Gln
            100                 105                 110
Ser Leu Gly Leu Leu Gly Asp Glu His Trp Ala Thr Asp Pro Asp Met
        115                 120                 125
Tyr Leu Gln Ser Pro Gln Ser Glu Arg Thr Asp Pro His Gly Leu Tyr
    130                 135                 140
Leu Ser Cys Asn Gly Gly Thr Pro Ala Gly His Lys Gln Met Pro Trp
145                 150                 155                 160
Pro Glu Pro Gln Ser Pro Arg Val Leu Pro Asn Gly Leu Ala Ala Lys
                165                 170                 175
Ala Gln Ser Leu Gly Pro Ala Glu Phe Gln Gly Ala Ser Gln Arg Cys
            180                 185                 190
Leu Gln Leu Gly Ala Cys Leu Gln Ser Ser Pro Pro Gly Ala Ser Pro
        195                 200                 205
Pro Thr Gly Thr Asn Arg His Gly Met Lys Ala Ala Lys His Gly Ser
    210                 215                 220
Glu Glu Ala Arg Pro Gln Ser Cys Leu Val Gly Ser Ala Thr Gly Arg
225                 230                 235                 240
Pro Gly Gly Glu Gly Ser Pro Ser Pro Lys Thr Arg Glu Ser Ser Leu
                245                 250                 255
Lys Arg Arg Leu Phe Arg Ser Met Phe Leu Ser Thr Ala Ala Thr Ala
            260                 265                 270
Pro Pro Ser Ser Ser Lys Pro Gly Pro Pro Pro Gln Ser Lys Pro Asn
        275                 280                 285
Ser Ser Phe Arg Pro Pro Gln Lys Asp Asn Pro Pro Ser Leu Val Ala
    290                 295                 300
Lys Ala Gln Ser Leu Pro Ser Asp Gln Pro Val Gly Thr Phe Ser Pro
305                 310                 315                 320
Leu Thr Thr Ser Asp Thr Ser Ser Pro Gln Lys Ser Leu Arg Thr Ala
                325                 330                 335
Pro Ala Thr Gly Gln Leu Pro Gly Arg Ser Ser Pro Ala Gly Ser Pro
            340                 345                 350
Arg Thr Trp His Ala Gln Ile Ser Thr Ser Asn Leu Tyr Leu Pro Gln
        355                 360                 365
Asp Pro Thr Val Ala Lys Gly Ala Leu Ala Gly Glu Asp Thr Gly Val
    370                 375                 380
Val Thr His Glu Gln Phe Lys Ala Ala Leu Arg Met Val Val Asp Gln
385                 390                 395                 400
Gly Asp Pro Arg Leu Leu Leu Asp Ser Tyr Val Lys Ile Gly Glu Gly
                405                 410                 415
Ser Thr Gly Ile Val Cys Leu Ala Arg Glu Lys His Ser Gly Arg Gln
            420                 425                 430
Val Ala Val Lys Met Met Asp Leu Arg Lys Gln Gln Arg Arg Glu Leu
        435                 440                 445
Leu Phe Asn Glu Val Val Ile Met Arg Asp Tyr Gln His Phe Asn Val
    450                 455                 460
Val Glu Met Tyr Lys Ser Tyr Leu Val Gly Glu Glu Leu Trp Val Leu
465                 470                 475                 480
```

```
Met Glu Phe Leu Gln Gly Gly Ala Leu Thr Asp Ile Val Ser Gln Val
                485                 490                 495
Arg Leu Asn Glu Glu Gln Ile Ala Thr Val Cys Glu Ala Val Leu Gln
            500                 505                 510
Ala Leu Ala Tyr Leu His Ala Gln Gly Val Ile His Arg Asp Ile Lys
        515                 520                 525
Ser Asp Ser Ile Leu Leu Thr Leu Asp Gly Arg Val Lys Leu Ser Asp
    530                 535                 540
Phe Gly Phe Cys Ala Gln Ile Ser Lys Asp Val Pro Lys Arg Lys Ser
545                 550                 555                 560
Leu Val Gly Thr Pro Tyr Trp Met Ala Pro Glu Val Ile Ser Arg Ser
                565                 570                 575
Leu Tyr Ala Thr Glu Val Asp Ile Trp Ser Leu Gly Ile Met Val Ile
            580                 585                 590
Glu Met Val Asp Gly Glu Pro Pro Tyr Phe Ser Asp Ser Pro Val Gln
        595                 600                 605
Ala Met Lys Arg Leu Arg Asp Ser Pro Pro Pro Lys Leu Lys Asn Ser
    610                 615                 620
His Lys Val Ser Pro Val Leu Arg Asp Phe Leu Glu Arg Met Leu Val
625                 630                 635                 640
Arg Asp Pro Gln Glu Arg Ala Thr Ala Gln Glu Leu Leu Asp His Pro
                645                 650                 655
Phe Leu Leu Gln Thr Gly Leu Pro Glu Cys Leu Val Pro Leu Ile Gln
            660                 665                 670
Leu Tyr Arg Lys Gln Thr Ser Thr Cys
        675                 680
```

<210> 4
<211> 545
<212> PRT
<213> Homo Sapiens

<400> 4

```
Met Ser Asn Asn Gly Leu Asp Ile Gln Asp Lys Pro Pro Ala Pro Pro
 1               5                  10                  15
Met Arg Asn Thr Ser Thr Met Ile Gly Val Gly Ser Lys Asp Ala Gly
            20                  25                  30
Thr Leu Asn His Gly Ser Lys Pro Leu Pro Pro Asn Pro Glu Glu Lys
        35                  40                  45
Lys Lys Lys Asp Arg Phe Tyr Arg Ser Ile Leu Pro Gly Asp Lys Thr
    50                  55                  60
Asn Lys Lys Lys Glu Lys Glu Arg Pro Glu Ile Ser Leu Pro Ser Asp
65                  70                  75                  80
Phe Glu His Thr Ile His Val Gly Phe Asp Ala Val Thr Gly Glu Phe
                85                  90                  95
Thr Gly Met Pro Glu Gln Trp Ala Arg Leu Leu Gln Thr Ser Asn Ile
            100                 105                 110
Thr Lys Ser Glu Gln Lys Lys Asn Pro Gln Ala Val Leu Asp Val Leu
        115                 120                 125
Glu Phe Tyr Asn Ser Lys Lys Thr Ser Asn Ser Gln Lys Tyr Met Ser
    130                 135                 140
Phe Thr Asp Lys Ser Ala Glu Asp Tyr Asn Ser Ser Asn Ala Leu Asn
145                 150                 155                 160
Val Lys Ala Val Ser Glu Thr Pro Ala Val Pro Pro Val Ser Glu Asp
                165                 170                 175
Glu Asp Asp Asp Asp Asp Asp Ala Thr Pro Pro Pro Val Ile Ala Pro
            180                 185                 190
Arg Pro Glu His Thr Lys Ser Val Tyr Thr Arg Ser Val Ile Glu Pro
        195                 200                 205
Leu Pro Val Thr Pro Thr Arg Asp Val Ala Thr Ser Pro Ile Ser Pro
    210                 215                 220
Thr Glu Asn Asn Thr Thr Pro Pro Asp Ala Leu Thr Arg Asn Thr Glu
225                 230                 235                 240
```

```
Lys Gln Lys Lys Lys Pro Lys Met Ser Asp Glu Glu Ile Leu Glu Lys
                245                 250                 255
Leu Arg Ser Ile Val Ser Val Gly Asp Pro Lys Lys Lys Tyr Thr Arg
            260                 265                 270
Phe Glu Lys Ile Gly Gln Gly Ala Ser Gly Thr Val Tyr Thr Ala Met
        275                 280                 285
Asp Val Ala Thr Gly Gln Glu Val Ala Ile Lys Gln Met Asn Leu Gln
    290                 295                 300
Gln Gln Pro Lys Lys Glu Leu Ile Ile Asn Glu Ile Leu Val Met Arg
305                 310                 315                 320
Glu Asn Lys Asn Pro Asn Ile Val Asn Tyr Leu Asp Ser Tyr Leu Val
                325                 330                 335
Gly Asp Glu Leu Trp Val Val Met Glu Tyr Leu Ala Gly Gly Ser Leu
            340                 345                 350
Thr Asp Val Val Thr Glu Thr Cys Met Asp Glu Gly Gln Ile Ala Ala
        355                 360                 365
Val Cys Arg Glu Cys Leu Gln Ala Leu Glu Phe Leu His Ser Asn Gln
    370                 375                 380
Val Ile His Arg Asp Ile Lys Ser Asp Asn Ile Leu Leu Gly Met Asp
385                 390                 395                 400
Gly Ser Val Lys Leu Thr Asp Phe Gly Phe Cys Ala Gln Ile Thr Pro
                405                 410                 415
Glu Gln Ser Lys Arg Ser Thr Met Val Gly Thr Pro Tyr Trp Met Ala
            420                 425                 430
Pro Glu Val Val Thr Arg Lys Ala Tyr Gly Pro Lys Val Asp Ile Trp
        435                 440                 445
Ser Leu Gly Ile Met Ala Ile Glu Met Ile Glu Gly Glu Pro Pro Tyr
    450                 455                 460
Leu Asn Glu Asn Pro Leu Arg Ala Leu Tyr Leu Ile Ala Thr Asn Gly
465                 470                 475                 480
Thr Pro Glu Leu Gln Asn Pro Glu Lys Leu Ser Ala Ile Phe Arg Asp
                485                 490                 495
Phe Leu Asn Arg Cys Leu Asp Met Asp Val Glu Lys Arg Gly Ser Ala
            500                 505                 510
Lys Glu Leu Leu Gln His Gln Phe Leu Lys Ile Ala Lys Pro Leu Ser
        515                 520                 525
Ser Leu Thr Pro Leu Ile Ala Ala Ala Lys Glu Ala Thr Lys Asn Asn
    530                 535                 540
His
545

      <210> 5
      <211> 525
      <212> PRT
      <213> Homo Sapiens

      <400> 5
Met Ser Asp Asn Gly Glu Leu Glu Asp Lys Pro Pro Ala Pro Pro Val
 1               5                  10                  15
Arg Met Ser Ser Thr Ile Phe Ser Thr Gly Gly Lys Asp Pro Leu Ser
            20                  25                  30
Ala Asn His Ser Leu Lys Pro Leu Pro Ser Val Pro Glu Glu Lys Lys
        35                  40                  45
Pro Arg His Lys Ile Ile Ser Ile Phe Ser Gly Thr Glu Lys Gly Ser
    50                  55                  60
Lys Lys Lys Glu Lys Glu Arg Pro Glu Ile Ser Pro Pro Ser Asp Phe
65                  70                  75                  80
Glu His Thr Ile His Val Gly Phe Asp Ala Val Thr Gly Glu Phe Thr
                85                  90                  95
Gly Met Pro Glu Gln Trp Ala Arg Leu Leu Gln Thr Ser Asn Ile Thr
            100                 105                 110
Lys Leu Glu Gln Lys Lys Asn Pro Gln Ala Val Leu Asp Val Leu Lys
        115                 120                 125
```

```
Phe Tyr Asp Ser Asn Thr Val Lys Gln Lys Tyr Leu Ser Phe Thr Pro
    130                 135                 140
Pro Glu Lys Asp Gly Leu Pro Ser Gly Thr Pro Ala Leu Asn Ala Lys
145                 150                 155                 160
Gly Thr Glu Ala Pro Ala Val Val Thr Glu Glu Glu Asp Asp Asp Glu
                165                 170                 175
Glu Thr Ala Pro Pro Val Ile Ala Pro Arg Pro Asp His Thr Lys Ser
            180                 185                 190
Ile Tyr Thr Arg Ser Val Ile Asp Pro Val Pro Ala Pro Val Gly Asp
        195                 200                 205
Ser His Val Asp Gly Ala Ala Lys Ser Leu Asp Lys Gln Lys Lys Lys
    210                 215                 220
Pro Lys Met Thr Asp Glu Glu Ile Met Glu Lys Leu Arg Thr Ile Val
225                 230                 235                 240
Ser Ile Gly Asp Pro Lys Lys Lys Tyr Thr Arg Tyr Glu Lys Ile Gly
                245                 250                 255
Gln Gly Ala Ser Gly Thr Val Phe Thr Ala Thr Asp Val Ala Leu Gly
            260                 265                 270
Gln Glu Val Ala Ile Lys Gln Ile Asn Leu Gln Lys Gln Pro Lys Lys
        275                 280                 285
Glu Leu Ile Ile Asn Glu Ile Leu Val Met Lys Glu Leu Lys Asn Pro
    290                 295                 300
Asn Ile Val Asn Phe Leu Asp Ser Tyr Leu Val Gly Asp Glu Leu Phe
305                 310                 315                 320
Val Val Met Glu Tyr Leu Ala Gly Gly Ser Leu Thr Asp Val Val Thr
                325                 330                 335
Glu Thr Ala Cys Met Asp Glu Ala Gln Ile Ala Ala Val Cys Arg Glu
            340                 345                 350
Cys Leu Gln Ala Leu Glu Phe Leu His Ala Asn Gln Val Ile His Arg
        355                 360                 365
Asp Ile Lys Ser Asp Asn Val Leu Leu Gly Met Glu Gly Ser Val Lys
    370                 375                 380
Leu Thr Asp Phe Gly Phe Cys Ala Gln Ile Thr Pro Glu Gln Ser Lys
385                 390                 395                 400
Arg Ser Thr Met Val Gly Thr Pro Tyr Trp Met Ala Pro Glu Val Val
                405                 410                 415
Thr Arg Lys Ala Tyr Gly Pro Lys Val Asp Ile Trp Ser Leu Gly Ile
            420                 425                 430
Met Ala Ile Glu Met Val Glu Gly Glu Pro Pro Tyr Leu Asn Glu Asn
        435                 440                 445
Pro Leu Arg Ala Leu Tyr Leu Ile Ala Thr Asn Gly Thr Pro Glu Leu
    450                 455                 460
Gln Asn Pro Glu Lys Leu Ser Pro Ile Phe Arg Asp Phe Leu Asn Arg
465                 470                 475                 480
Cys Leu Glu Met Asp Val Glu Lys Arg Gly Ser Ala Lys Glu Leu Leu
                485                 490                 495
Gln His Pro Phe Leu Lys Leu Ala Lys Pro Leu Ser Ser Leu Thr Pro
            500                 505                 510
Leu Ile Met Ala Ala Lys Glu Ala Met Lys Ser Asn Arg
        515                 520                 525
```

<210> 6
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 6
gccccgaatt cggcaccatg ttccgcaag 29

<210> 7

<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 7
ctcgtggcgg ccgctcagca ggtggaggtc tg 32

<210> 8
<211> 681
<212> PRT
<213> Artificial Sequence

<220>
<223> Example Dominant Negative Mutant Embodiment of SEQ ID NO:3

<400> 8
Met Phe Arg Lys Lys Lys Lys Lys Arg Pro Glu Ile Ser Ala Pro Gln
1 5 10 15
Asn Phe Gln His Arg Val His Thr Ser Phe Asp Pro Lys Glu Gly Lys
20 25 30
Phe Val Gly Leu Pro Pro Gln Trp Gln Asn Ile Leu Asp Thr Leu Arg
35 40 45
Arg Pro Lys Pro Val Val Asp Pro Ser Arg Ile Thr Arg Val Gln Leu
50 55 60
Gln Pro Met Lys Thr Val Val Arg Gly Ser Ala Met Pro Val Asp Gly
65 70 75 80
Tyr Ile Ser Gly Leu Leu Asn Asp Ile Gln Lys Leu Ser Val Ile Ser
85 90 95
Ser Asn Thr Leu Arg Gly Arg Ser Pro Thr Ser Arg Arg Arg Ala Gln
100 105 110
Ser Leu Gly Leu Leu Gly Asp Glu His Trp Ala Thr Asp Pro Asp Met
115 120 125
Tyr Leu Gln Ser Pro Gln Ser Glu Arg Thr Asp Pro His Gly Leu Tyr
130 135 140
Leu Ser Cys Asn Gly Gly Thr Pro Ala Gly His Lys Gln Met Pro Trp
145 150 155 160
Pro Glu Pro Gln Ser Pro Arg Val Leu Pro Asn Gly Leu Ala Ala Lys
165 170 175
Ala Gln Ser Leu Gly Pro Ala Glu Phe Gln Gly Ala Ser Gln Arg Cys
180 185 190
Leu Gln Leu Gly Ala Cys Leu Gln Ser Ser Pro Pro Gly Ala Ser Pro
195 200 205
Pro Thr Gly Thr Asn Arg His Gly Met Lys Ala Ala Lys His Gly Ser
210 215 220
Glu Glu Ala Arg Pro Gln Ser Cys Leu Val Gly Ser Ala Thr Gly Arg
225 230 235 240
Pro Gly Gly Glu Gly Ser Pro Ser Pro Lys Thr Arg Glu Ser Ser Leu
245 250 255
Lys Arg Arg Leu Phe Arg Ser Met Phe Leu Ser Thr Ala Ala Thr Ala
260 265 270
Pro Pro Ser Ser Ser Lys Pro Gly Pro Pro Pro Gln Ser Lys Pro Asn
275 280 285
Ser Ser Phe Arg Pro Pro Gln Lys Asp Asn Pro Pro Ser Leu Val Ala
290 295 300
Lys Ala Gln Ser Leu Pro Ser Asp Gln Pro Val Gly Thr Phe Ser Pro
305 310 315 320
Leu Thr Thr Ser Asp Thr Ser Ser Pro Gln Lys Ser Leu Arg Thr Ala
325 330 335
Pro Ala Thr Gly Gln Leu Pro Gly Arg Ser Ser Pro Ala Gly Ser Pro

```
                340                      345                      350
Arg Thr Trp His Ala Gln Ile Ser Thr Ser Asn Leu Tyr Leu Pro Gln
            355                 360                 365
Asp Pro Thr Val Ala Lys Gly Ala Leu Ala Gly Glu Asp Thr Gly Val
    370                 375                 380
Val Thr His Glu Gln Phe Lys Ala Ala Leu Arg Met Val Val Asp Gln
385                 390                 395                 400
Gly Asp Pro Arg Leu Leu Leu Asp Ser Tyr Val Lys Ile Gly Glu Gly
                405                 410                 415
Ser Thr Gly Ile Val Cys Leu Ala Arg Glu Lys His Ser Gly Arg Gln
            420                 425                 430
Val Ala Val Arg Met Met Asp Leu Arg Lys Gln Gln Arg Arg Glu Leu
        435                 440                 445
Leu Phe Asn Glu Val Val Ile Met Arg Asp Tyr Gln His Phe Asn Val
    450                 455                 460
Val Glu Met Tyr Lys Ser Tyr Leu Val Gly Glu Glu Leu Trp Val Leu
465                 470                 475                 480
Met Glu Phe Leu Gln Gly Gly Ala Leu Thr Asp Ile Val Ser Gln Val
                485                 490                 495
Arg Leu Asn Glu Glu Gln Ile Ala Thr Val Cys Glu Ala Val Leu Gln
            500                 505                 510
Ala Leu Ala Tyr Leu His Ala Gln Gly Val Ile His Arg Asp Ile Lys
        515                 520                 525
Ser Asp Ser Ile Leu Leu Thr Leu Asp Gly Arg Val Lys Leu Ser Asp
    530                 535                 540
Phe Gly Phe Cys Ala Gln Ile Ser Lys Asp Val Pro Lys Arg Lys Ser
545                 550                 555                 560
Leu Val Gly Thr Pro Tyr Trp Met Ala Pro Glu Val Ile Ser Arg Ser
                565                 570                 575
Leu Tyr Ala Thr Glu Val Asp Ile Trp Ser Leu Gly Ile Met Val Ile
            580                 585                 590
Glu Met Val Asp Gly Glu Pro Pro Tyr Phe Ser Asp Ser Pro Val Gln
        595                 600                 605
Ala Met Lys Arg Leu Arg Asp Ser Pro Pro Pro Lys Leu Lys Asn Ser
    610                 615                 620
His Lys Val Ser Pro Val Leu Arg Asp Phe Leu Glu Arg Met Leu Val
625                 630                 635                 640
Arg Asp Pro Gln Glu Arg Ala Thr Ala Gln Glu Leu Leu Asp His Pro
                645                 650                 655
Phe Leu Leu Gln Thr Gly Leu Pro Glu Cys Leu Val Pro Leu Ile Gln
            660                 665                 670
Leu Tyr Arg Lys Gln Thr Ser Thr Cys
        675                 680
```

```
<210> 9
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 9
caggtggccg tccggatgat ggacctc                                        27

<210> 10
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer
```

<400> 10
gaggtccatc atccggacgg ccacctg 27

<210> 11
<211> 681
<212> PRT
<213> Artificial Sequence

<220>
<223> Example Dominant Negative Mutant Embodiment of SEQ ID NO:3

<400> 11
```
Met Phe Arg Lys Lys Lys Lys Lys Arg Pro Glu Ile Ser Ala Pro Gln
1               5                   10                  15
Asn Phe Gln Leu Arg Val Leu Thr Ser Phe Asp Pro Lys Glu Gly Lys
            20                  25                  30
Phe Val Gly Leu Pro Pro Gln Trp Gln Asn Ile Leu Asp Thr Leu Arg
        35                  40                  45
Arg Pro Lys Pro Val Val Asp Pro Ser Arg Ile Thr Arg Val Gln Leu
    50                  55                  60
Gln Pro Met Lys Thr Val Val Arg Gly Ser Ala Met Pro Val Asp Gly
65                  70                  75                  80
Tyr Ile Ser Gly Leu Leu Asn Asp Ile Gln Lys Leu Ser Val Ile Ser
                85                  90                  95
Ser Asn Thr Leu Arg Gly Arg Ser Pro Thr Ser Arg Arg Arg Ala Gln
            100                 105                 110
Ser Leu Gly Leu Leu Gly Asp Glu His Trp Ala Thr Asp Pro Asp Met
        115                 120                 125
Tyr Leu Gln Ser Pro Gln Ser Glu Arg Thr Asp Pro His Gly Leu Tyr
    130                 135                 140
Leu Ser Cys Asn Gly Gly Thr Pro Ala Gly His Lys Gln Met Pro Trp
145                 150                 155                 160
Pro Glu Pro Gln Ser Pro Arg Val Leu Pro Asn Gly Leu Ala Ala Lys
                165                 170                 175
Ala Gln Ser Leu Gly Pro Ala Glu Phe Gln Gly Ala Ser Gln Arg Cys
            180                 185                 190
Leu Gln Leu Gly Ala Cys Leu Gln Ser Ser Pro Pro Gly Ala Ser Pro
        195                 200                 205
Pro Thr Gly Thr Asn Arg His Gly Met Lys Ala Ala Lys His Gly Ser
    210                 215                 220
Glu Glu Ala Arg Pro Gln Ser Cys Leu Val Gly Ser Ala Thr Gly Arg
225                 230                 235                 240
Pro Gly Gly Glu Gly Ser Pro Ser Pro Lys Thr Arg Glu Ser Ser Leu
                245                 250                 255
Lys Arg Arg Leu Phe Arg Ser Met Phe Leu Ser Thr Ala Ala Thr Ala
            260                 265                 270
Pro Pro Ser Ser Ser Lys Pro Gly Pro Pro Pro Gln Ser Lys Pro Asn
        275                 280                 285
Ser Ser Phe Arg Pro Pro Gln Lys Asp Asn Pro Pro Ser Leu Val Ala
    290                 295                 300
Lys Ala Gln Ser Leu Pro Ser Asp Gln Pro Val Gly Thr Phe Ser Pro
305                 310                 315                 320
Leu Thr Thr Ser Asp Thr Ser Ser Pro Gln Lys Ser Leu Arg Thr Ala
                325                 330                 335
Pro Ala Thr Gly Gln Leu Pro Gly Arg Ser Ser Pro Ala Gly Ser Pro
            340                 345                 350
Arg Thr Trp His Ala Gln Ile Ser Thr Ser Asn Leu Tyr Leu Pro Gln
        355                 360                 365
Asp Pro Thr Val Ala Lys Gly Ala Leu Ala Gly Glu Asp Thr Gly Val
    370                 375                 380
Val Thr His Glu Gln Phe Lys Ala Ala Leu Arg Met Val Val Asp Gln
385                 390                 395                 400
```

```
Gly Asp Pro Arg Leu Leu Leu Asp Ser Tyr Val Lys Ile Gly Glu Gly
                405                 410                 415
Ser Thr Gly Ile Val Cys Leu Ala Arg Glu Lys His Ser Gly Arg Gln
            420                 425                 430
Val Ala Val Arg Met Met Asp Leu Arg Lys Gln Gln Arg Arg Glu Leu
        435                 440                 445
Leu Phe Asn Glu Val Val Ile Met Arg Asp Tyr Gln His Phe Asn Val
    450                 455                 460
Val Glu Met Tyr Lys Ser Tyr Leu Val Gly Glu Glu Leu Trp Val Leu
465                 470                 475                 480
Met Glu Phe Leu Gln Gly Gly Ala Leu Thr Asp Ile Val Ser Gln Val
                485                 490                 495
Arg Leu Asn Glu Glu Gln Ile Ala Thr Val Cys Glu Ala Val Leu Gln
            500                 505                 510
Ala Leu Ala Tyr Leu His Ala Gln Gly Val Ile His Arg Asp Ile Lys
        515                 520                 525
Ser Asp Ser Ile Leu Leu Thr Leu Asp Gly Arg Val Lys Leu Ser Asp
    530                 535                 540
Phe Gly Phe Cys Ala Gln Ile Ser Lys Asp Val Pro Lys Arg Lys Ser
545                 550                 555                 560
Leu Val Gly Thr Pro Tyr Trp Met Ala Pro Glu Val Ile Ser Arg Ser
                565                 570                 575
Leu Tyr Ala Thr Glu Val Asp Ile Trp Ser Leu Gly Ile Met Val Ile
            580                 585                 590
Glu Met Val Asp Gly Glu Pro Pro Tyr Phe Ser Asp Ser Pro Val Gln
        595                 600                 605
Ala Met Lys Arg Leu Arg Asp Ser Pro Pro Pro Lys Leu Lys Asn Ser
    610                 615                 620
His Lys Val Ser Pro Val Leu Arg Asp Phe Leu Glu Arg Met Leu Val
625                 630                 635                 640
Arg Asp Pro Gln Glu Arg Ala Thr Ala Gln Glu Leu Leu Asp His Pro
                645                 650                 655
Phe Leu Leu Gln Thr Gly Leu Pro Glu Cys Leu Val Pro Leu Ile Gln
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Leu Tyr Arg Lys Gln Thr Ser Thr Cys
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